

A COMPARISON OF THE WEIGHT OF THE INTESTINE WITH THE BODY AND KIDNEY WEIGHTS IN RATS WHICH WERE FED ARTIFICIAL, UNBALANCED DIETS ¹

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It would seem reasonable to expect some alteration of the intestine when the physiologic load is increased, just as the heart and the kidney hypertrophy when more work is thrown upon those organs. For testing that hypothesis a group of animals were fed unbalanced, markedly different diets, and the intestine studied for changes, post-mortem. It was essential, however, that the diets employed be nutritionally adequate; otherwise the study would have been an investigation in malnutrition or starvation. The usual signs of health such as normal growth rate, smoothness of coat, brightness of eyes, fertility, and physical activity were noted. The work performed by the intestine has been divided into two phases — digestion and absorption. Anatomically the intestine is divided into two parts, large and small, but the work is shared jointly although not equally by the two parts. Theoretically a diet may affect structurally the whole intestine, or one of its parts.

Experimental studies on the effect of diet upon the intestine of fish, amphibia, and birds have been reported by Yung (1883, '09), Gulland (1898), Lonnberg ('02), Weiss ('01), Houssay ('02), Babàk ('06), Roux ('06), Schepelman ('06), and Elven ('28). These studies sprung primarily from the

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observations of de Cuvier and Milne-Edwards that the intestine was longer in herbivorous than in carnivorous animals. In addition, search for proof for Lamarck's theory of "use and disuse" was stimulating morphological investigation.

Another interest in the problem developed later when advances in the medical sciences acted as a stimulus to research in the relationship of diet and growth. In addition, the establishment of the value and importance of vitamins in the diet was of the greatest assistance in interpreting the conflicting findings in earlier investigations.

Wetzel ('28) studied the relation of the weight and length of the intestine to the body in rats which had been fed plant, meat, and mixed diets. The plant and mixed diets were fed to three, the meat diet to 7 rats. The plant diet consisted of wheat (sometimes corn) supplemented occasionally with oats, rye, barley, fresh fruits, nuts, and germinated peas. The meat diet consisted of raw beef alternated with raw horse meat, including the bones; occasionally raw frog muscle was supplied. Fresh milk was given daily. The mixed diet was a combination of the meat and plant diets. He found that the large intestine was heavier on the plant diet than on either the meat or the mixed diet, while the small intestine was lighter than on either of the other two diets. In the rats on the mixed diet both the small and large intestine were intermediate in weight.

Addis ('32) reported measurements on the intestine of rats which had been fed a concentrated diet or a contrasting bulky diet which contained 40% dry alfalfa and 10% dry agar by weight, for 44 weeks. He found a 48% increase in the weight of the large intestine, and a 10% increase in the weight of the small intestine on the bulky compared with the weight on the concentrated diet. These percentages were based on the area of the body surface. The reported increase on the bulky diet is, however, not to be interpreted as a real increase because the body surface on the bulky diet was considerably less than the body surface on the concentrated diet.

Wierda ('42) reported that rats which had been fed diets containing raw liver had a longer small intestine than rats which were fed a meat-free diet. However, rats which were fed raw beef or cooked dried beef showed no significant increase in the length of the small intestine. It was also reported that the length of the colon was not increased by the addition of meat to the diet. Rats which had been fed a diet containing from 10 to 30% finely ground agar showed no increase in the length of the small intestine; however, the caecum and colon were definitely longer than in the concentrated diet rats. The surface area of both small (excluding the villi) and large intestine was greater on the agar and meat diet than on the concentrated diet but the surface of the large intestine was definitely greater on the agar diet. The mucosa of the small intestine showed a definite response to the meat diet, the villi being large, high, wide and thick, and differing in these respects from the villi on the balanced concentrated diet.

Localized overgrowths of the wall of the colon were found in rats on a high-fat diet (Wierda, '43). The great size of the overgrowths which assumed the shape of diverticula indicated the existence of an exceedingly high growth potentiality. A similar finding was reported by Carlson and Hoelzel ('49) using a bulky diet.

MATERIALS AND METHODS

In these experiments 50 albino rats, including an equal number of each sex (Wistar strain) were maintained for 44 weeks on 4 different diets beginning at the time of weaning (see table 1). The animals were housed in screen-bottom cages and both food and water were kept continuously before the animals. The agar was finely ground, so that neither it nor any of the other ingredients could be separated from the diet by the rats. The salt was a modification of the Osborne-Mendel salt mixture (Wesson, '32). The rats used were the offspring of 10 matings, and each litter was spread over the 4 dietary groups as far and as evenly as possible so that

any hereditary differences would be distributed equally. At the end of the feeding period the rats were deprived of food for 24 hours before they were asphyxiated with coal-gas and autopsied. Each rat was weighed and its intestine carefully detached, slit open, and washed free from its contents. It was

TABLE 1
*Average of measurements, with probable error, of 50 rats after 44 weeks
of experimental feeding*

NO. OF ANIMALS	COMPOSITION OF DIET		GROSS BODY WEIGHT	WEIGHT OF TOTAL FAT	WEIGHT OF SMALL INTESTINE	WEIGHT OF LARGE INTESTINE
14	Balanced		257 gm	16 gm	3.21 gm	1.83 gm
	Concentrated		± 10	± 1.9	± 0.08	± 0.04
	Casein	18%				
	Lard	23				
	Cornstarch	53				
	Salt mixture	4				
12	Bulky		261 gm	29 gm	3.76 gm	3.31 gm
	Casein	18%	± 11	± 1.4	± 0.21	± 0.17
	Lard	23				
	Cornstarch	23				
	Agar	30				
	Salt mixture	4				
12	Carbohydrate		268 gm	29 gm	4.26 gm	1.85 gm
	Casein	14%	± 12	± 6.8	± 0.24	± 0.06
	Cornstarch	83				
	Salt mixture	4				
12	Fat		278 gm	41 gm	3.86 gm	1.74 gm
	Casein	25%	± 11	± 5.2	± 0.15	± 0.07
	Lard	69				
	Salt mixture	6				

The diet of each rat was supplemented daily with 5 drops of cod liver oil and 0.4 gm of dry yeast.

then dried lightly with filter paper, placed in a stoppered weighing bottle, and weighed. The kidney was also carefully removed, dried by blotting with filter paper, and weighed in a stoppered weighing bottle. Finally the whole carcass was analyzed for fat using a modification of the Rose-Gottlieb

method for fat determination. The method consisted of saponification of each rat separately with potassium hydroxide, after which the volume was brought up to two liters, using a calibrated volumetric flask. An aliquot (10 cm³) was transferred to a Röhrig tube and the fat separated by washing three times with 25 and 15 cm³ of ether and petroleum ether. The combined washings were then freed from ether by evaporation on a steam bath to a constant weight. From this value the total amount of fat in the rat was calculated.

RESULTS

The averages of the measurements taken are shown in table 1. The average body weight at autopsy was nearly the same on the 4 diets. The greatest variation was between the balanced concentrated diet (257 gm) and the fat diet (278 gm). The gross weight on the bulky and concentrated diets was intermediate — 261 gm on the bulky diet and 268 gm on the carbohydrate diet. When the significance of these differences was tested statistically by the method of the probable error of the difference of the mean, it was found that there was no significant difference in gross weight. The probability was only equal that a difference in weight existed between the rats on the balanced concentrated diet that weighed the least and the fat diet rats that weighed the most.

The average amount of fat in the body (cellular and extracellular) at autopsy was least on the balance concentrated diet (16 gm) and greatest on the fat diet (41 gm), while on both the bulky and the carbohydrate diets the amount was 29 gm. When these figures were tested for validity by the method of the probable error of the difference of the mean it was found that it was a practical certainty that the fat diet rats possessed a greater amount of fat than the balanced concentrated diet rats. There was no significance to the difference in the amount of fat in the rats on the other diets.

The weight of the body of the rats, exclusive of fat, ranged less widely than the gross weight. The range of the net weight was from 241 gm on the balanced concentrated diet

to 232 gm on the bulky diet. In body tissues which were not saponifiable there was a spread of only 4% among the 4 groups.

The small intestine was heaviest on the carbohydrate diet, and lightest on the balanced concentrated diet. The difference in weight was 32%. The weight of the small intestine was on the average nearly the same on the fat diet and the bulky diet, and was not significantly less than on the carbohydrate diet, nor greater than on the balanced concentrated diet. The average weight of the small intestine on the 4 diets was 3.77 gm. On the balanced concentrated diet the weight of the small intestine was 17% less than the average of all 4 dietary groups; on the carbohydrate diet it was 11% greater; on the fat diet it was 2% greater, and on the bulky diet it was very nearly identical with the average. When these differences were checked by the probable error of the difference of the mean it was found that the probability was high that the small intestine was heavier on the carbohydrate than on the balanced concentrated diet. The probability was low for a real difference in weight between the other groups.

The large intestine was considerably heavier on the bulky diet than on the three contrasting (concentrated) diets. When the absolute weights were compared, it was found that the large intestine was 78% heavier on the bulky diet than on the carbohydrate diet; 80% heavier than on the balanced concentrated diet; and 90% heavier than on the fat diet. The difference between the weight of the large intestine on the bulky diet and on the three contrasting diets was great enough to make it highly probable that the large intestine was heavier on the bulky than on any of the other diets. When this test for significance was applied to the differences in weight of the large intestine on the carbohydrate, balanced concentrated, and fat diets, it was found that the probability was low that a real difference existed.

The average weight of the kidneys was greatest on the carbohydrate diet and least on the bulky diet. The difference in weight between these two groups was 27%, a difference

which when tested statistically indicated a high probability of a real difference. The average weight of the kidneys on the balanced concentrated diet was 17% less than on the carbohydrate diet, and on the fat diet the kidneys weighed 11% less than on the carbohydrate diet. But the kidneys of the balanced concentrated, fat, and carbohydrate diet animals were not significantly different in weight.

Table 2 shows the average weight of the kidneys in grams, and the ratio of the weight of the small and large intestine to the kidney weight after feeding the experimental diets for 44 weeks. On the balanced concentrated diet the small intestine was 155% of the weight of the kidneys; on the bulky

TABLE 2

The average weight of the kidneys in grams, with probable error; and the ratio of the weight of the small and large intestine to kidney weight after feeding the experimental diets for 44 weeks

TYPE OF DIET	KIDNEY WEIGHT	SMALL INTESTINE	LARGE INTESTINE
Balanced concentrated	2.07 $\pm$ 0.07	1.55	0.88
Bulky	1.91 $\pm$ 0.08	1.96	1.73
Carbohydrate	2.44 $\pm$ 0.10	1.74	0.75
Fat	2.19 $\pm$ 0.09	1.76	0.79

diet 196%; and on the carbohydrate and fat diet 174% and 176% respectively. In terms of kidney weight the small intestine was on the average 26% heavier on the bulky diet than on the balanced concentrated diet; and 12% heavier than on either the carbohydrate or fat diet.

The large intestine on the bulky diet was 173% heavier than the kidneys; on the balanced concentrated diet 88% heavier; on the carbohydrate diet 75% heavier; and on the fat diet 79% heavier. In terms of kidney weight the large intestine was 96% heavier on the bulky than on the balanced concentrated diet; 130% heavier than on the carbohydrate diet; and 118% heavier than on the fat diet.

DISCUSSION

The purpose of this experiment was to determine the effect of indigestible material in the diet upon the weight of the small and large intestine. Four diets were employed, one of which was a bulky diet containing a relatively large amount of agar—a poorly digestible substance, and three were contrasting, concentrated diets which contained no such ingredient but varied in respect to materials furnishing the calories. Of the three concentrated diets the fat diet had the highest caloric value—7.21 calories per gram; the carbohydrate diet 3.88; and the balanced concentrated and bulky diets each had 4.91. To satisfy the caloric requirement it was necessary for the rats on the bulky diet to eat 4 times more material than the rats on the fat diet because dry agar is very bulky and has a low caloric value. Furthermore after the agar becomes wet it swells so that actually the intestine of the bulky diet animals handled approximately 10 times more material than the intestine of the rats on the fat diet. The carbohydrate and balanced concentrated diets did not swell upon wetting. Nevertheless, 4.5 times more material was handled by the intestine on the carbohydrate, and 5 times more on the balanced concentrated than on the fat diet. Wetzel ('28) did not calculate the relative amount of material handled by the intestine of his experimental animals, but Addis ('32) estimated that the intestine of his bulky diet rats handled 4 times more material (by volume) than the intestine of the "smooth" (concentrated) diet rats. Wetzel dealt particularly with the problem of the effect of meat, and of plant diets upon the weight and length of segments of the intestine, as well as the whole tract.

Both Wetzel ('28) and Addis ('32) encountered difficulty in interpreting the observed weight of the intestine on the contrasted diets because of the difference in body size of the rats. In Wetzel's experiments the difference was not great, but he believed that it accounted for the relatively heavier small intestine on the "plant" diet compared with the "flesh" diet. In the experiments of Addis the body weight of the

bulky diet rats was not much more than half that of the "smooth" (concentrated) diet animals, consequently no direct comparison of the weight of the intestine was made, but its weight was compared with the body surface. (Using the formula — body surface = 11.3×0.666 body weight.)

In the present experiments the rats weighed approximately the same, at autopsy, on the 4 diets. This fact indicates that agar can compose as much as 30% of the diet of the rat without a resultant decrease in the growth rate of the body compared with the artificial diets containing no bulky ingredient. The fat determination shows that only the fat diet rats contained significantly more fat than the bodies of the leanest, i.e., the concentrated diet rats. The weight of the non-fat part of the rats was nearly the same on each of the diets, a fact which shows further that the potentiality for growth was not inhibited by any of the diets.

At autopsy the rats which had been fed the carbohydrate diet had a small intestine which exceeded the average weight on the other diets, and was also significantly heavier (statistically) than in the rats which had been fed the balanced concentrated diet. It is not clearly seen why there is this contrast in effect between these two diets. According to the theory of Roux ('06) a concentrated diet, rich in easily digestible substances, is capable of stimulating the growth of the small intestine, thereby enabling a greater and more rapid digestion and absorption of the food, but in these experiments the bulky diet (30% agar) rats showed a heavier small intestine, at autopsy, than the rats which had been fed a concentrated balanced diet. Such a finding is plainly not in accord with Roux's theory — for the balanced concentrated diet is clearly more easily digested than the bulky diet. According to Wetzel ('28) and Wierda ('42) the feeding of a meat diet or a diet containing meat resulted in a longer and heavier small intestine in rats than a plant diet, or a diet containing casein as the source of protein. Addis ('32), however, reported finding an 8% increase in the weight of the small intestine on a bulky diet, but since this calculation is

based on the body surface of rats which weighed only half as much as the controls, there is reason to doubt the existence of a real increase.

The weight of the large intestine at autopsy clearly shows that hypertrophy has occurred on the bulky diet. For although the body size was nearly the same as on the concentrated diets, the large intestine was 78% heavier than on the carbohydrate diet; 80% heavier than on the balanced concentrated diet; and 90% heavier than on the fat diet. These values are considerably higher than Addis ('32) found. He reported only a 48% increase in weight for the large intestine but the contrast in his diets was less than in these experiments and, in addition, his bulky diet animals were only half the size of those on the concentrated diet.

The weights of the kidneys were compared with the weight of the small and large intestine because the kidneys have been used frequently as a criterion for enlargement of other organs, although it has been shown by several investigators that the coefficient of correlation of kidney weight with body weight is very high. In the rat on a stock diet values of 0.96 and 0.95 were reported for males and females respectively (Webster, Liljegren and Zimmers, '47). In terms of kidney weight the small intestine was found to be heaviest on the bulky diet, exceeding the weight on the concentrated balanced diet by 26% and both the carbohydrate and fat diets by 12%. The large intestine was likewise heaviest on the bulky diet in terms of kidney weight, exceeding the weight on the balanced concentrated diet by 96%, and on carbohydrate and fat diet by 130 and 118% respectively. The observed weight of the large intestine after 44 weeks of feeding a bulky diet indicates an enlargement of approximately the same magnitude as Flint ('12) observed in dogs from whom 50 to 70% of the small intestine had been removed surgically. No gross changes in the large intestine, such as diverticula, were observed in any of these rats but there was a marked increase in caliber (diameter) as well as in the thickness of the wall.

In aged rats hypertrophy of the colon through the development of diverticula has been reported by Wierda ('43) and Carlson and Hoelzel ('49).

SUMMARY

A group of 50 albino rats were fed an artificial unbalanced diet beginning at the time of weaning and continuing for 44 weeks, to determine the effect upon the weight of the small and large intestine. The animals were asphyxiated with coal-gas after having been starved for 24 hours. The small and large intestine and the kidneys were then carefully isolated, trimmed free from attached tissue, and weighed using glass stoppered weighing bottles to prevent dehydration. The carcass was saponified and the amount of fat in it was determined by a modification of Rose-Gottlieb method for fat determination. The weight of the small and large intestine on the 4 diets were then compared with each other and with the body (gross, and minus fat) and kidney weight.

The gross weight of the rats was found to be not significantly different on the 4 diets; however, those which had received the fat diet possessed a significantly greater amount of fat than those which had received the balanced concentrated diet. The net weight of the body (gross weight minus fat) was even more nearly the same on the 4 diets than the gross weight.

The small intestine was found to be heaviest on the carbohydrate diet and lightest on the balanced concentrated diet. This difference was great enough to be statistically significant; the average difference was 32%. The small intestine was intermediate in weight between these two extremes on the fat and bulky diets. These differences in weight cannot be accounted for on the basis of the concentration of the diet. The heavier weight of the intestine on the carbohydrate diet may be due to the ease with which the diet is digested, and the rapidity with which it moves through the small intestine, but the balanced concentrated diet differs hardly enough from the carbohydrate diet to account for the lightness in weight of

the small intestine on the balanced concentrated diet. The large intestine was significantly heavier in the rats which had been fed the bulky diet than in those which had received the concentrated diets. The average weight was 78% greater than on the carbohydrate diet; 80% greater than on the balanced concentrated diet; and 90% greater than on the fat diet.

In terms of kidney weight the small intestine was on the average 26% heavier on the bulky diet than on the balanced concentrated diet, and 12% heavier than on either the carbohydrate or fat diet. The large intestine was, in terms of kidney weight, 96% heavier on the bulky diet than on the balanced concentrated diet; 130% heavier than on the carbohydrate diet; and 118% heavier than on the fat diet. The increased weight of the colon was not due to diverticula, but primarily to an increase in diameter, and a thickening of the wall.

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A NOTE ON THE CAROTID BODY AND CAROTID SINUS OF VARANUS MONITOR

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THREE FIGURES

The present work was prompted by Boyd's ('42) study of the nerve supply of the branchial arch arteries in *Vipera berus*, and it was thought advisable to study the innervation and characteristics of this region in other reptiles also. The first to be studied was *Varanus* and incidentally it revealed many interesting features which throw light on the probable evolution of the carotid sinus-carotid gland complex.

MATERIAL AND METHODS

Six adult *Varanus monitor* were dissected providing 12 specimens for study. The terminal part of the common carotid artery along with the commencement of the internal and external carotid arteries was removed in one piece. The specimens were then washed free of blood in .6% NaCl solution. Six specimens were fixed in Bouin's fluid, cut serially and stained with haematoxylin and eosin. The remaining 6 specimens, also sectioned serially, were stained by the Biel-schowsky's technique.

OBSERVATIONS AND COMMENTS

Gross examination of the fresh specimens showed an elongated fusiform swelling, pinkish in colour, on the dorsal side of the arteries, extending from near the termination of the common carotid artery, involving the bifurcation, and continuing up the internal carotid artery for a variable distance.

On an average the fusiform area was 7.2 mm long and 3.4 mm at its greatest diameter.

Examination of serial sections showed that the lumen of the vessel was significantly dilated at the termination of the common carotid artery, the dilatation extending up to and involving the commencement of the internal carotid artery.

The lumen of the vessel was uniform in outline, there being no irregularities as are present in *Ophidia* (Boyd, '42) or emerald lizard (Palme, '34). The most significant feature of the sections, however, was the presence of a large mass of epithelioid cells on the dorsal aspect of the vessel, localised in the region of the carotid sinus. The presence of epithelioid cells in the walls of the branchial arteries has been described both by Boyd ('42) and Palme ('34), but they have not mentioned any regular arrangement of these cells. In *Varanus monitor*, on the other hand, we find that the greater part of the fusiform swelling, seen on naked eye examination, is made up of an aggregation of these cells on the dorsal wall. These cells lie enmassed between the adventitia and the media of the vessel, and from the latter fibromuscular trabeculae extend outwards and break up this cellular mass into separate loculi. Each smaller region thus marked out is occupied by closely packed columns of epithelioid cells about 5 to 8 rows in thickness. These cells are more or less polygonal in shape and take up the eosin stain rather faintly. They possess a very distinct, centrally placed, round nucleus, with a well marked nucleolus in each.

Through the middle of each such loculus there runs a blood sinusoid filled with the characteristic nucleated red blood cells. These blood sinusoids at places open into the lumen of the carotid artery and their endothelium is directly continuous with that of the sinus region of the artery.

Figure 1 is a microphotograph of a transverse section across the bifurcation of the common carotid artery under the low power of the microscope. It shows the aggregation of epithelioid cells with fibromuscular trabeculae passing between the columns of cells.

The nerve supply of this area is from the glossopharyngeal nerve by a twig which passes caudally in between the internal and external carotid arteries and enters the portion of the media which separates the epithelioid cell mass from the lumen of the carotid sinus. The nerve rapidly breaks up into

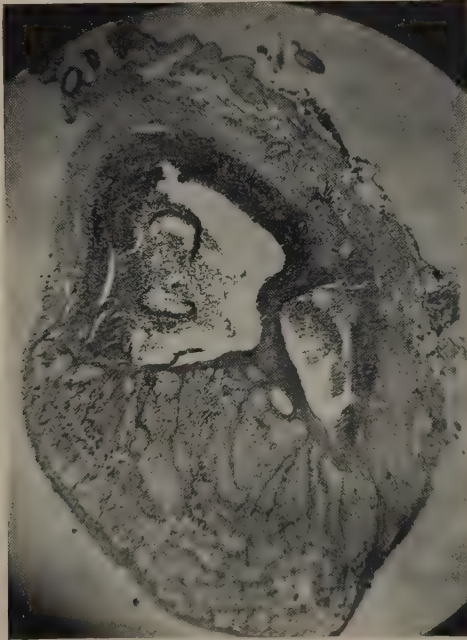


Fig. 1 Transverse section across carotid gland of *Varanus monitor*, stained with haematoxylin and eosin. The two arteries seen in section are the internal and external carotid arteries. The epithelioid cell mass is present on one side of the bifurcation.

a number of fine filaments which go partly to supply the media of the arterial wall and partly to the epithelioid cell mass. As in *Ophidia* the fibres terminating in the wall of the vessel are only slightly branched. The nerve fibres destined for the epithelioid cell mass pass along the fibromuscular trabeculae and finally, breaking up into finer filaments end among the

cells. Figure 2 shows nerve fibres passing in one such trabecula in the midst of epithelioid cells.

It is worthy to note that no pigment cells of any kind are present in this region in *Varanus monitor* in contradistinction to the descriptions in *Ophidia* and *Amphibia*.

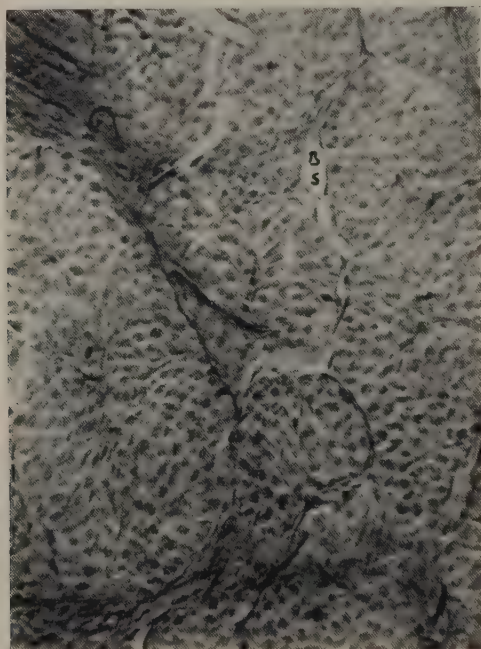


Fig. 2 High power microphotograph of epithelioid cells of carotid gland of *Varanus monitor* (Bielschowsky's silver impregnation), showing nerve fibres traversing the cell mass. At BS is a blood sinusoid.

The nature of the so-called epithelioid cells has been discussed at length by Boyd ('42): "Until more definite evidence on the precise origin and histogenesis of these cells is available it seems more satisfactory to use a non-committal term for them. Glomus cells or epithelioid cells are, at present, more satisfactory as terms of reference for them than para-

ganglionic cells." Again, Boyd states: "Leaving as undetermined, then, the nature of the cells described by Trinci, Palme and Adams in the neighbourhood of the commencement of the reptilian internal carotid artery, the presence of the thickening in the wall of the artery, and of the nerve endings in and around this thickening, enables one to state that the reptilian third arch arteries have a specialised nervous apparatus associated with them . . ."

According to the above argument the structure in *Varanus monitor* has evolved even more since (a) the lumen of the carotid sinus region is quite uniform in outline, (b) the adventitial coat is not as markedly thickened as Boyd has pointed out in *Ophidia*, and (c) the epithelioid cells which are present as irregular collections in the adventitia in *Amphibia* (Palme, '34) (and not definitely mentioned by Boyd in his paper) are aggregated into a well defined mass, fit to be called a carotid body, to which distinct nerve fibres are distributed, and in which blood sinusoids lie in between the rows of cells.

A comparison of figure 3, the carotid body of a cat, with figure 1 will help to appreciate how the carotid body of *Varanus monitor* approaches very nearly the mammalian organ in structure. In the cat, the carotid body is applied to the dorsal aspect of the internal carotid artery, its connective tissue stroma being directly continuous with the adventitia of the artery. The parenchymal cells are situated in groups around sinusoids. In *Varanus monitor* also we find the epithelioid cells arranged around blood sinusoids, and here the relation of the carotid body to the arterial wall is more intimate inasmuch as the connective tissue trabeculae are continuous with the media of the artery. Evidently the carotid sinus-carotid gland apparatus subserving pressoreceptive and chemoreceptive functions are very intimately blended together in the lower vertebrates and as we ascend higher (due to greater specialisation of function?) the two organs dissociate from one another.

The structure of the carotid gland and carotid sinus in *Varanus monitor* is distinctly a stage midway between that described by Boyd in *Ophidia* and the structure in mammals.

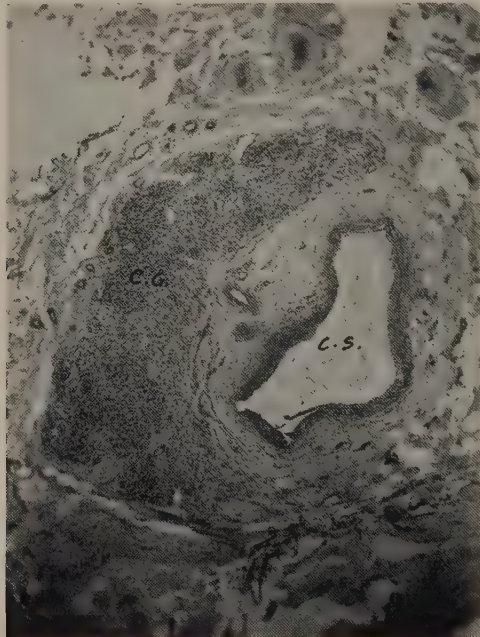


Fig. 3 Low power microphotograph of carotid gland of cat showing intimate relationship of the carotid gland (CG) and carotid sinus (CS).

SUMMARY

The carotid gland and carotid sinus of *Varanus monitor* is described with special reference to their structural affinities with the *Ophidia* on the one hand and the mammals on the other. The carotid sinus is present in the form of a dilatation of the lumen of the termination of the common carotid artery and commencement of the internal carotid artery, the walls of the sinus being uniform in outline. The carotid gland consists of a fusiform aggregation of epithelioid cells applied to

the dorsal aspect of the carotid sinus, and partially subdivided into separate loculi by fibromuscular trabeculae which radiate from the media of the artery into this mass. Within the group of cells are blood sinusoids. Nerve fibres are found to terminate as free nerve terminals both in the wall of the carotid sinus and in the fibromuscular trabeculae dividing up the epithelioid cell mass.

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ELECTROMYOGRAPHIC STUDIES OF M. BICEPS BRACHII DURING NORMAL VOLUNTARY MOVEMENT AT THE ELBOW

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TWO FIGURES

INTRODUCTION

The increased interest in muscles in recent years has served to focus attention on the paucity of good information on the behavior of muscles in the normal human individual during voluntary movements. A few individuals using different techniques have made contributions that are referred to frequently in the literature. The study of the response of individual muscles to electrical stimulation is perhaps highlighted by the work of Duchenne (1867) recently published in translation (Kaplan, '49). The analysis of muscles and joints on a purely mechanical basis was carried to a high level by Fick ('10, '11). The study by direct observation and palpation was very successful in the hands of Beevor ('03) and Wright ('28). There is a long and excellent literature on the properties of muscles removed from the body and in the experimental animal. Only in rather recent years has the study of muscles in the normal human individual through electromyography become at all common. While this offers many difficulties of interpretation it does give information that advances, but does not complete the picture of muscle function. An excellent discussion of the problems of the interpretation of the electromyogram has been given by Adrian ('25) and by Denny-Brown ('49), who, in addition, has contributed an adequate review of the literature.

Our purpose was to determine whether in a given movement a particular muscle was active and whether it was equally active throughout a movement, or whether it was more active during one phase of a movement. Appreciating that many variables enter into the picture when one uses surface electrodes it seemed important to know whether there was a "pattern" of potentials for a specific muscle under a given set of conditions, whether the pattern was much the same for different individuals and whether it was similar for the same individual at different times.

The term "pattern" is of necessity a loose one but there are in each record areas of higher and lower deflections variable in location and extent and perhaps the term covers the overall picture as well as any word can. It may be permissible to enumerate a few obvious things as a background for examining the records. Each muscle is made up of many units not all of which are necessarily active at the same time. In most instances there are several muscles capable of producing a given movement. In this situation no single muscle is necessarily active throughout the entire movement or if active throughout it need not be equally active in all phases. It must also be appreciated that there are factors of summation and cancellation and that chance must of necessity enter into the picture. There are undoubtedly subjective factors that that it is not possible to eliminate. Repeated experiments on the same individual does suggest however that there is a fair amount of regularity in relative amplitudes which can best be expressed by short verbal comments and the free use of illustrations.

SUBJECTS AND METHODS

Electromyograms were obtained from 8 normal subjects. All were students and faculty in the department of anatomy.

The electromyographs used were modified electrocardiographs. The basic unit was an early model electrocardiograph (Model A) manufactured by the General Electric Company. This photographic recording machine proved very satisfactory. Its viewing screen enabled the operator to determine if

conditions were satisfactory for a recording. The sensitivity of this unit was increased approximately 10-fold by the inclusion of an amplifier in the subject circuit. The modification made it necessary to work within a screened room. Interference was further reduced by a copper woven wire screen between the operator and the electromyograph. Most of the records were made at the speed customarily used for electrocardiographs (2.5 cm a second) but adjustment of the governor allowed film speeds up to 5.0 cm a second. A switch in the light circuit provided a means of interrupting the recording briefly at any point and thus was useful as a marker at the beginning and end of the movement. Three electromyographs were arranged so that they could be activated simultaneously.

Covered wire leads were used between the surface electrodes and the added amplifier. Electrodes were zinc disks 1.0 cm in diameter. These were held in place on the subject's skin by a liquid adhesive.

Action potentials from *m. biceps brachii* of the right side were led off through a pair of electrodes from each of the two heads. Markings for the placement of the electrodes were made with the biceps in full contraction. The vertical distance between the two electrodes forming the pair was 3.0 cm; the horizontal distance between the pairs was approximately 3.0 cm. The muscle belly was thus divided, for recording purposes, into a lateral portion continuous with the long head and a medial portion continuous with the short head. All records were taken with the subject in the sitting position. In the starting position for the flexion movements the arm was at the side in slight lateral rotation as in the anatomical position and in contact near the elbow with a bar which prevented extension of the arm beyond the plane of the trunk. The forearm was either in supination or in pronation. When the subject was relaxed in this position the muscle was essentially electrically negative. The starting position for the extension movements differed in that the elbow joint was flexed so that the angle between the arm and forearm was

about 45° and the biceps, instead of being electrically negative, showed moderate action potentials. When the operator was convinced, by observation of the galvanometer beam, that conditions were satisfactory for recording he signalled the subject to begin the movement. The subject signalled the operator at the end of the movement. The arm position for the holds was the same as for the movements; the elbow was in a position of partial flexion and the forearm in supination. There were of course potentials present as soon as the hold position was assumed.

Records were obtained during flexion (acceleration), hold (zero acceleration) and extension (negative acceleration) at the elbow. Flexion and extension were performed without additional load and with loads of 1.0 kg and 2.0 kg, with the forearm supine and with the forearm prone. The positions of partial flexion for the hold records were such that the angles between the arm and forearm were 135° , 90° , and 45° during three successive records. These were made without load and with a load of 1.0 kg.

RESULTS

Flexion — acceleration at the elbow

All of the electromyograms showed deflections which begin at or near the initiation of the movement and continue during the entire excursion of the forearm. The pattern of these deflections without added load (record 1) showed a gradual increase in amplitude during the first third or half of the record followed by an irregular plateau which was maintained to the end of the movement.

When a load of 1.0 or 2.0 kg was carried in the hand an overall increase in amplitude resulted (2, 3). The most conspicuous increase in amplitude occurred in the middle portion of the record when the movements were made with the forearm supine (2, 3).

The effect of the position of an accessory joint was demonstrated by the recording of a lower amplitude when the forearm was prone (4, 5, 6, 7).

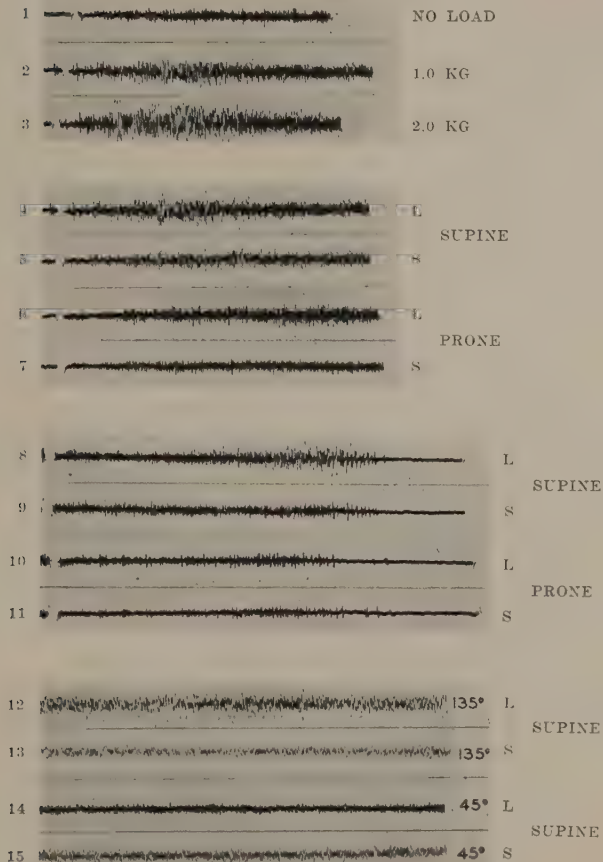


Fig. 1 Records from the same subject during one experiment.

1-3 Elbow flexion (acceleration), forearm supine, long head. Recording time for record 1 was 5.2 seconds. These records illustrate the effect of added load.

4-7 Elbow flexion (acceleration), added load 1.0 kg. These records illustrate the relative activity of the long head (L) and the short head (S) and the effect on the activity of the biceps when accessory joints (radioulnar), crossed by the muscle, are changed in position.

8-11 Elbow extension (deceleration) added load 1.0 kg. The general amplitude is less than in the acceleration series. The period of essentially zero potential is an interesting feature of the deceleration movement.

12-15 Hold (zero acceleration) added load 1.0 kg. These records illustrate the variable effect of the degree of flexion at the elbow on the activity of the biceps. The amplitude may be essentially the same as shown by the records of short head (13, 15) or different as shown by the records of the long head (12-14).

Usually the amplitude of the recording from the long head was slightly greater than that from the short head (4, 5), but the maximum amplitude for the series was recorded from the short head with the forearm supine and an added load of 2.0 kg.

When simultaneous recordings from the long and short heads were compared in detail the electromyograms of some movements were similar in pattern (6, 7). This similarity occurred more frequently when the forearm was in the prone position.

Extension — negative acceleration at the elbow

All of the electromyograms showed deflections of low to moderate amplitude preceding the movement because in the starting position the elbow joint was flexed with an angle of 45° between the arm and the forearm. The pattern of the electromyogram for this series was variable but the most frequent pattern can be divided into three areas. The first portion of the record shows a relatively low but fairly uniform amplitude; the second portion is a region of increased amplitude which builds up gradually and decreases more abruptly; the final portion is essentially at zero potential.

Of the records made without load only the recording from the long head with the forearm supine was characteristic of the above description. The rest of this series showed a relatively uniform, low amplitude over the first two-thirds of the record followed by zero potentials during the final third.

When added load was held in the hand the characteristic pattern occurred more frequently (8). Added load also produced a general increase in amplitude and a shortening of that portion showing zero potentials.

The effect of the position of the forearm was demonstrated by a lower amplitude when the forearm was prone (8, 9, 10, 11) and was most obvious in the 2.0 kg series.

The potentials recorded from the long head were greater than those from the short head during each movement in the deceleration series.

Hold — zero acceleration at the elbow

The electromyograms showed continuous activity whenever the muscle served to hold the forearm against the pull of gravity (12, 13, 14, 15). The individual deflections were irregular in amplitude, but usually were distributed in a manner which gave the total recording a relatively uniform appearance. Some of the records showed groups of 3-10 deflections of higher amplitude irregularly distributed.

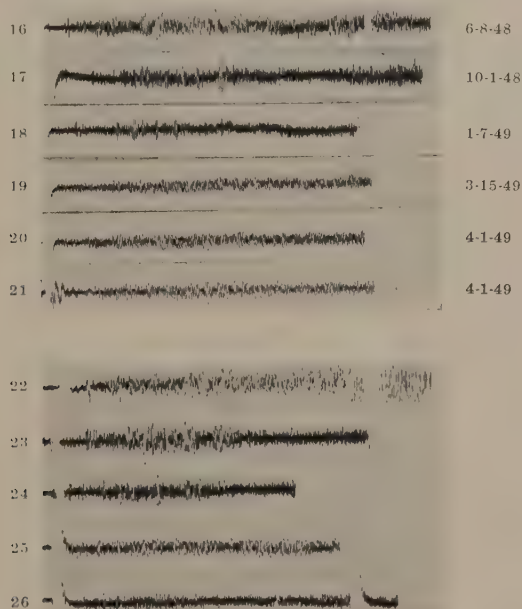


Fig. 2 Records of the same movement from a single subject at irregular intervals.

16-21 Flexion (acceleration) at elbow, forearm supine, short head, added load 1.0 kg. The patterns of activity shown in 16 and 17 differ from the others in having a secondary rise in potentials in the last part of the movement. The general amplitude is greater in the first two records than in the later ones. Total times for record 17: 7.4." The short interruption in 16 indicates the end of the movement. There is a brief recording of hold potentials after that.

22-26 Records of the same movement from different subjects.

Flexion (acceleration) at elbow, forearm supine, long head, added load 1.0 kg.

In many trials the activity was greatest in the 135° position (12, 14). In other trials the amplitude was essentially the same in all three positions.

Recordings from the same individual

Electromyograms were recorded under similar conditions and from the same subject at irregular intervals over a period of 10 months. These tracings showed variations in both amplitude and pattern (16-21). There was a progressive decrease in amplitude in each succeeding trial for the first three of the 6 trials. The last three trials were approximately equal in amplitude to the third. Records 16 and 17 are different in pattern from the others in the series in that each showed an area of higher deflections near the end.

Recordings from different individuals

Electromyograms of the same movement recorded from different subjects varied in both amplitude and pattern. The degree of variation in amplitude may be as much as three-fold for equivalent areas of comparable records (22 and 26). A difference in pattern was obvious in some instances (22, 23) and not obvious in others (23, 24).

SUMMARY

Action potentials were recorded from m. biceps brachii of normal subjects during voluntary movements of the elbow joint. Repeated recordings from one subject at the same sitting were usually similar. Repeated recordings from one subject with intervals of weeks intervening usually, but not always, gave similar patterns. Action potentials tended to be higher on the earlier trials than on the later ones. When two or more subjects were studied under similar conditions the recordings were similar or different in pattern and amplitude. The variations in pattern and amplitude were assumed to represent differences in the use of the muscle during a given movement. Potentials of lower amplitude were recorded from

both parts of the muscle when the movement was performed with the forearm in the prone position and, in general, from the short head under all conditions studied.

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EFFECTS OF THYROXIN INJECTIONS ON GROWTH AND DIFFERENTIATION OF THE SKELETON OF HYPOPHYSECTOMIZED FEMALE RATS¹

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SEVEN FIGURES

The purpose of this experiment was to study the effects of thyroxin injections on skeletal growth and differentiation in hypophysectomized rats.

As noted in a previous paper (Ray, Simpson, Li, Asling and Evans, '50) the results of experiments conducted at the Institute of Experimental Biology (Evans, Simpson and Pencharz, '39; Becks, Ray, Simpson and Evans, '42) and those conducted by Laqueur, Dingemanse and Freud ('41) have been at variance. The Amsterdam group have reported that they were able to obtain growth in hypophysectomized rats with "thyranon pro injectione" (N. V. Organon in Oss), a proteolytic degradation product of thyroid gland. This substance was injected at a daily dose of 2.5 to 5 μ g of organic bound iodine. The exact thyroxin equivalent of this dosage cannot be computed. The increase of weight of 8 hypophysectomized male rats during three weeks of injections averaged 16 gm. Repeated experiments by the Berkeley group, on the other hand, have failed to confirm this finding. The essential difference between the experiments conducted by the two groups may have been the dose level of thyroid hor-

¹ Aided by grants from the United States Public Health Service Grant RG-409 (C2) and the Research Board of the University of California.

mone injected — that used at Berkeley being from 5 to 7 μg of thyroxin per day.² Several recent experiments (Scow et al., '49; Ray et al., '50) have shown that the optimum dose of thyroxin in thyroparathyroidectomized rats was from 2.5 to 3.0 μg a day. Accordingly in the present experiment it was decided to use the same dose in hypophysectomized rats to determine if the effects were different from those observed at the higher dose levels.

METHOD

Ten female rats of the Long-Evans strain were hypophysectomized at 21 days of age. Five of the animals were injected daily with thyroxin. The other five served as uninjected controls. A third group of three unoperated uninjected animals of the same chronological age was included as normal controls. During the experimental period, each group of animals was kept in a single heated cage, and all were fed the same diet. During the last 17 hours of the experimental period, the total food consumption of the animals for each of the three groups was determined.

Crystalline thyroxin (Squibb) was used, dissolved in a slightly alkaline aqueous solution. Three micrograms were injected subcutaneously daily. Injections were started at a chronological age of 30 days and continued for 30 days.

The animals were weighed every 5 days. At 30, 46, and 60 days of age each animal was anesthetized and roentgenograms taken. From the films the skeletal age and the length of the various parts of the skeletons were determined. At 61 days of age, the animals were autopsied. The tibia, humerus, third metacarpal and third costochondral junction were removed for histological study and, in addition, the right femur was preserved for chemical analysis. The technique of prepara-

² In a recent study (Asling, Becks, Simpson, and Evans, '49) prolongation of the injection period to 71 days at the 5 μg /day dose level resulted in body lengths of the injected animals which exceeded those of the hypophysectomized controls by a slight, statistically significant amount. Body weights remained the same as in the controls.

tion of the histological sections and method of gravimetric analysis for water, fat, organic content and ash have been described in a previous report (Ray et al., '50).

RESULTS

During the experimental period the animals receiving thyroxin were more active and had better appetities than the uninjected hypophysectomized rats. The total food intake for the injected group during the last 17 hours of the experimental period was 6.33 gm/rat as compared with 4.33 gm/rat for the uninjected group.

The average skeletal age, body weight and body length determinations are given in table 1.

The thyroxin-injected rats not only ate more but also gained more weight than the uninjected animals, the mean weight gain during the experimental period being 20 gm for the injected rats as opposed to 9 gm for the hypophysectomized controls. As can be seen from figure 1, the gain in weight of the injected group occurred during the last 20 days of the experimental period.³

The thyroxin-injected rats also significantly exceeded their uninjected controls in body length (table 1).

In addition to the increase in length of the axial skeleton, measurements from the roentgenograms of representative bones of the appendicular skeleton revealed an increase in length following thyroxin injections in the hypophysectomized rats. The figures for the ulna, tibia, and the 6th caudal vertebra are given in table 2.

It is instructive to compare the growth of these two groups of rats with the skeletal maturation which they attained. As shown in table 1, the skeletal age of the uninjected hypophysectomized rats was 42 days at 60 days of chronologic age.

³ The best growth only approached the unit of growth (1 gm per day) during the last part of the experimental period. Even here it was only demonstrated by using a dose lower than those previously employed, whereas it is a characteristic of the hypophyseal growth hormone that increasing doses give proportionate increases in body weight.

TABLE 1
Estimated skeletal age, and average body weight and length of hypophysectomized female rats injected with thyroxin¹

GROUP	NO. OF RATS	SKELETAL AGE		BODY WEIGHT		BODY LENGTH AT AUTOPSY		
		At 30 days	At 60 days	At autopsy	Gain during injection period	Nose to anus	Anus to tail-tip	Total
		days	days	gm	gm	mm	mm	mm
Hypophysectomized, uninjected	5	29 ± 0.9 ²	42 ± 1.3	65 ± 2.4	9 ± 1.2	134 ± 2.0	112 ± 2.8	246 ± 4.0
Hypophysectomized, thyroxin, 3.0 µg per day	5	29 ± 1.4	55 ± 2.2	78 ± 2.5	20 ± 1.4	147 ± 1.3	125 ± 3.1	272 ± 3.9
Intact, uninjected	3	30 ± 0.0	60 ± 0.0	152 ± 11.7	85 ± 10.0	185 ± 5.1	164 ± 5.9	349 ± 10.8

¹ Hypophysectomized when 21 days of age; injected subcutaneously with thyroxin from 30 to 60 days of age.

² Average of 4 rats.

TABLE 2
Average lengths of ulna, tibia, and 6th caudal vertebra of hypophysectomized female rats injected with thyroxin¹

GROUP	NO. OF RATS	ULNA			TIBIA			6TH CAUDAL VERTEBRA		
		At 30 days	At 46 days	At 60 days	At 30 days	At 46 days	At 60 days	At 30 days	At 46 days	At 60 days
		mm	mm	mm	mm	mm	mm	mm	mm	mm
Hypophysectomized, uninjected	5	20.9 ² ± 0.17	21.4 ± 0.25	21.7 ± 0.26	24.4 ² ± 0.26	25.3 ± 0.24	25.9 ± 0.30	5.2 ² ± 0.10	5.5 ± 0.13	5.7 ± 0.08
Hypophysectomized, thyroxin, 3.0 µg per day	5	21.1 ± 0.10	22.0 ± 0.14	22.8 ± 0.08	24.6 ± 0.21	26.8 ± 0.22	28.0 ± 0.22	5.2 ± 0.13	6.0 ± 0.08	6.3 ± 0.16
Intact, uninjected	3			26.9 ± 0.53			32.4 ± 0.54		8.6 ± 0.12	

¹ Hypophysectomized when 21 days of age; measurements made on roentgenograms taken at intervals during the injection of thyroxin from 30 to 60 days of age.

² Average of 4 rats.

Their size (body weight, length, and tibia length) resembled that of much younger rats, approximately 30 days of age. This indicated that growth was depressed by hypophysectomy to a greater extent than was skeletal maturation. The thyroxin-injected rats had skeletal ages of 55 days at 60 days chronological age, though their bodily dimensions were like those of 33-day old normal rats. This indicated that the thyroxin stimulated skeletal maturation more than it stimulated growth.

Since the main basis for the roentgenographic criteria of skeletal age was the degree of development of the secondary

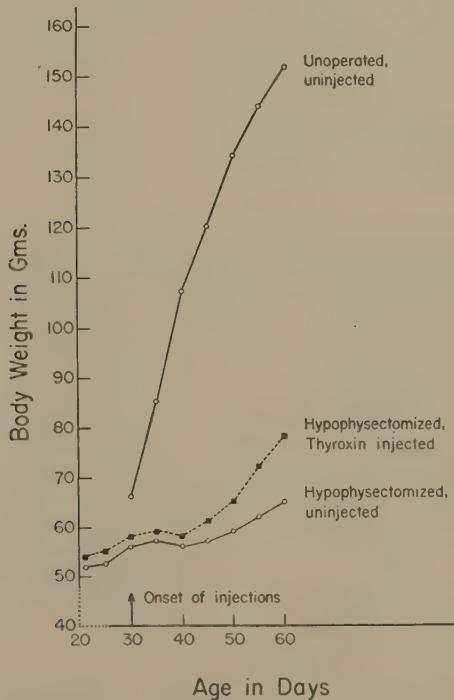


Fig. 1 Curve showing the average growth of 5 hypophysectomized rats injected with thyroxin compared with that of 5 uninjected hypophysectomized and three normal rats during the 30-day experimental period.

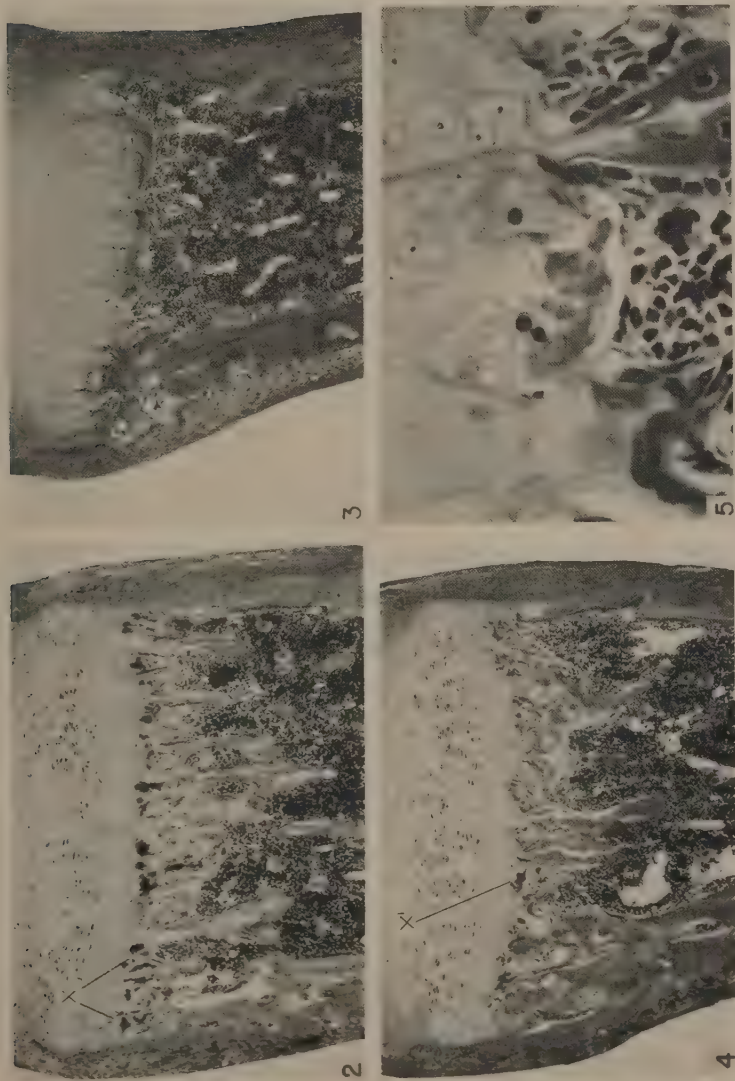
(epiphyseal) ossification centers, the histogenetic means by which this advance in skeletal differentiation occurred was of special interest. Several regions of endochondral ossification (proximal end of tibia, third metacarpal, distal end of humerus, and costochondral junction) were therefore sectioned for histological study.

In the tibia, the main interest was in the width and activity of its proximal epiphyseal plate. If differentiation were progressing faster than proliferation, the plate should be narrower than in the controls (i.e., cartilage removal should be more rapid than cartilage formation). Such a narrowing was actually observed, but in this experiment it did not become statistically significant.⁴ The width in the thyroxin-injected rats was $258 \pm 0.73 \mu$, compared with $273 \pm 22.5 \mu$ in the hypophysectomized controls, and $275 \pm 28.9 \mu$ in the normal rats. Histologically the thyroxin-injected rats showed more marrow in contact at the erosion zone of the cartilage plate than did the hypophysectomized controls, but did not approach the normal rats in this respect.

Premature epiphyseal closure was not found at the metacarpal epiphysis. The roentgenographic estimate of the skeletal age (55 days) at 60 days of chronological age did not justify one in expecting closure, since the normal time of closure is 90–110 days (Becks, Asling, Collins, Simpson and Evans, '48).

Histologic support of the roentgenographic indications of skeletal aging was gained from study of the distal end of the humerus. In the hypophysectomized controls fragments of the main epiphyseal plate still remained at 60 days of age, although normally these are all removed by 40 days of age (Becks, Asling, Simpson, Evans and Li, '48). In the thyroxin-injected rats all such fragments had been removed. In one animal of this group, premature closure of the medial

⁴In a study to be reported with C. P. Williams, thyroxin injections starting immediately after hypophysectomy and continuing for 40 days did result in a significant reduction of the cartilage plate.



Figs. 2-5 Third left costochondral junction of 60-day old female rats. Frontal sections, Mallory stain, $\times 80$, (except as otherwise stated).
 Fig. 2 Normal. (Sp. 813)
 Fig. 3 Hypophysectomized at 21 days of age, no injections. (Sp. 808)
 Fig. 4 Hypophysectomized at 21 days of age, injected with $3 \mu\text{g}$ of thyroxin daily from 30 to 60 days of age. (Sp. 803)
 Fig. 5 Higher magnification ($\times 475$) of multinucleated chondroblast shown at X' in figure 4.

epicondylar epiphysis of the humerus was found; normally this cartilage remains intact until 102–135 days of age.

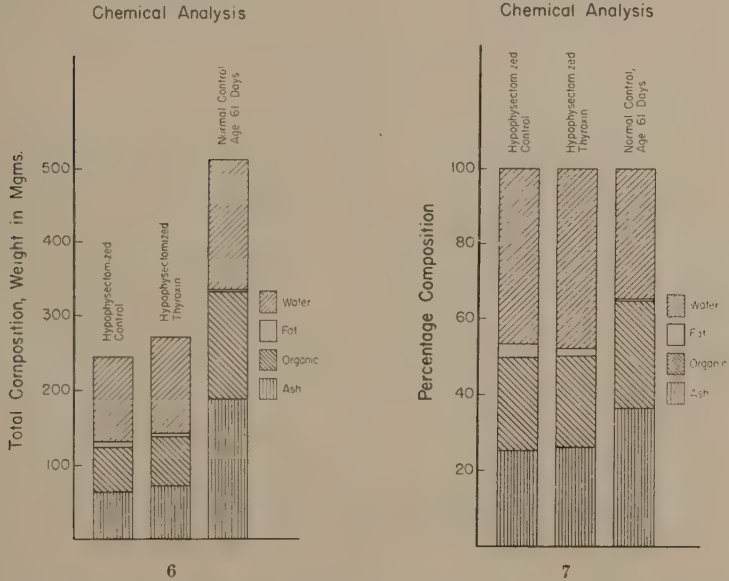
The most marked evidence of excessive cartilage erosion was observed at the costochondral junction (figs. 2–5). Here the hypophysectomized controls showed complete cessation of endochondral ossification, the cartilage margin being sealed from the marrow cavity by bone. The injections of thyroxin produced slightly greater proliferation of cartilage, and also prevented its sealing-off by bone. Marrow contact and cartilage erosion was maintained. The marrow tufts were more cellular than vascular.

Figures 2 to 5 illustrate the costochondral junction in a normal rat, a hypophysectomized control, and a thyroxin-injected hypophysectomized rat. Figure 2 shows a balance between cartilage formation, cartilage erosion, and osteogenesis characteristic of normal endochondral bone growth. At the top of the picture small nests of reserve chondrocytes are seen. Next, in the zone of proliferating cells the flattened chondrocytes are found disposed in rows and conical clusters. Finally there may be seen two or three layers of enlarged, rounded chondrocytes with a vacuolated cytoplasm and faint-staining nuclei. Tufts of marrow are in contact with these cells along a regular margin, the erosion line. Blood-filled capillaries are seen in most of these tufts. Although not shown in detail at this magnification the tufts often contain large, irregularly shaped, multinucleated cells. Two such are indicated by the lines marked X. Deeper in the marrow space the elongated bony trabeculae of the spongiosa are seen, at first straight and delicate but subsequently anastomosing. In the primary spongiosa most of the trabeculae contain cores of cartilage matrix, continuous with the longitudinal partitions of the cartilage in the zone of enlarged cells.

In figure 3 the cartilage in the untreated hypophysectomized rat shows poor alignment of cells. A layer of bone seals the cartilage from the marrow cavity. No bony trabeculae are seen.

Figure 4 illustrates the response to injection of thyroxin. The cartilage is more like that in normal rats. Abundant marrow is in contact with the cartilage. Osteogenesis is active, although the arrangement of trabeculae is not as regular as in normal rats. An important feature shown in these rats is the presence of many chondroclasts at the line of cartilage erosion. One such cell is seen at X', and is shown in these rats in higher magnification in figure 5. These cells are multinucleated, have a vacuolated cytoplasm, and are ir-

regularly shaped. Often the vertical striations of the "brush border" may be seen against the matrix. They resemble osteoclasts in all respects save their contact with cartilage instead of bone. They were seen more clearly in the injected hypophysectomized rats than in normal rats; in the latter, although present in the marrow tufts, they were often obscured by the capillaries accompanying them. In the uninjected hypophysectomized rats they were scarce.



Figs. 6 and 7 Chemical composition of the femur of hypophysectomized rats uninjected and injected with thyroxin, and of normal rats of the same age (60 days).

Fig. 6 Composition by weight.

Fig. 7 Percentage composition.

Chemical analysis of the femur did not demonstrate any difference in the bones of thyroxin-injected and untreated hypophysectomized rats. Their water, fat, remaining organic material, and ash contents are shown by graphs in figure 6 (average weight, in milligrams) and figure 7 (percentage composition). Both groups showed relatively greater water

content, and less organic material and ash than in the group of normal animals. The calcium and phosphorus contents of the ash were similar in all three groups of rats.⁵ Expressed in milligrams per 100 mg of ash, the calcium values were 40.2 ± 0.75 , 39.7 ± 1.04 , and 37.8 ± 0.10 for the uninjected hypophysectomized, the thyroxin-injected hypophysectomized, and the normal rats, respectively. The corresponding values for phosphorus were 16.6 ± 0.84 , 17.2 ± 0.12 , and 16.7 ± 0.20 , respectively.

SUMMARY

A study of the effect of thyroxin in producing skeletal growth and differentiation in hypophysectomized female rats was conducted by comparing body dimensions and roentgenographic, histologic, and chemical analyses of the skeletons with those of untreated hypophysectomized and normal rats.

The untreated rats (hypophysectomized at 21 days of age and sacrificed at 60 days of age) showed marked retardation of growth in body weight and length, and a delay, though less pronounced, in skeletal maturation (judged roentgenographically). The hypophysectomized rats treated with thyroxin ($3.0 \mu\text{g}/\text{day}$ between 30 and 60 days of age) showed an increase in appetite and a slight gain in body weight and body length over the hypophysectomized untreated rats. On the other hand, there was a marked gain in skeletal maturation.

Histologic studies of the proximal end of the tibia, third metacarpal, distal end of humerus, and costochondral junction showed arrest of endochondral ossification in the uninjected hypophysectomized rats. In the thyroxin-injected rats, erosion of cartilage and replacement by bone continued, though chondrogenesis was not maintained at an equal rate. This accounted for the greater differentiation of their skeletons demonstrated roentgenographically.

⁵ We acknowledge gratefully the assistance of Dr. Harold D. Copp, Division of Physiology, University of California, in the analyses of calcium and phosphorus in the bone-ash samples.

On chemical analysis of the femur, both uninjected and injected hypophysectomized rats had more water and less organic matter and ash than normal. No evidence of chemical maturation of the skeleton was therefore found.

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A MORPHOLOGICAL AND CYTOCHEMICAL STUDY OF THE POSTNATAL DEVELOPMENT OF THE RAT'S ADRENAL CORTEX¹

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SIXTEEN FIGURES

This paper reports the results of a histological and cytochemical study of the adrenal cortex in the albino rat during development from birth to 6 weeks of age. The observations refer primarily to the question of the zonation of secretory activity in the cortex, as affected by the growth and reorganization of the gland after birth. Zonation of the cortex is of particular interest because studies on the adult rat have suggested that while both the zona glomerulosa and zona fasciculata secrete steroid hormones, they serve different functions and are separately controlled (Deane, Shaw and Greep, '48; Greep and Deane, '49a). Earlier studies on the cortex of the young rat (Jackson, '19; Engström, '36; Tobin and Whitehead, '42; and Mitchell, '48) indicate that zonation of the cortex, with respect to both histological organization and lipid content, is present from birth. These investigators were not concerned with the functional separation of the two zones, however, nor did they attempt to judge whether the cortex is actively secreting in infancy. We have therefore used cytochemical techniques designed to reveal hormone precursors (Deane, Shaw and Greep). A morphological study of these adrenals was made also so that our series of animals could be compared with those of the earlier reports.

¹ This work was done in part under a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

In addition, because of the difference of opinion concerning the occurrence of a fetal cortex or X zone in the rat adrenal gland (Howard, '38; Tobin and Whitehead), attention has been paid to any signs which might distinguish the inner part of the cortex from the outer zones.

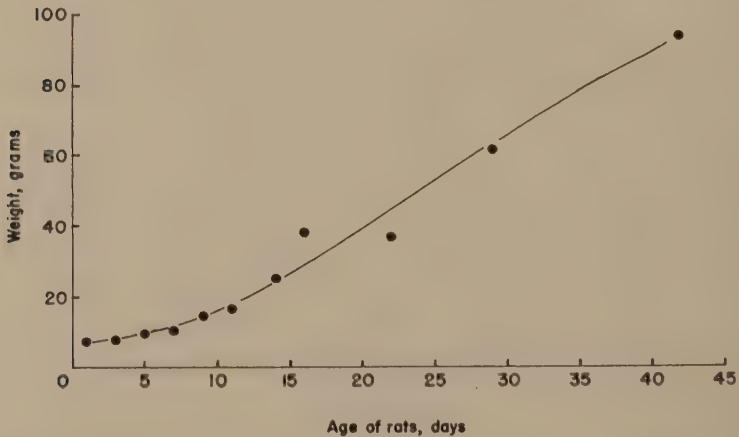


Fig. 1 Each dot represents the average weight of the 4 young rats at the time they were killed. The approximate growth curve of the animals has been drawn in.

MATERIAL AND METHODS

Rats of the Sprague-Dawley strain that were fed Purina laboratory chow were used. Following breeding, female rats were placed in separate cages. The number of young in each litter was reduced to 6 immediately after birth. The mother was left with her young for 30 days.

Four young rats were killed at each of the following ages: 1, 3, 5, 7, 9, 11, 14, 16, 22, 29 and 42 days. For each age group, two animals were taken from one litter, two from another—in each instance one male and one female. The average weights of the rats used are presented in figure 1.

The rats were killed by decapitation. One adrenal gland from each was fixed overnight in Bouin's fluid, dehydrated,

embedded in paraffin, and sectioned serially at 10 μ . The sections were stained with Harris' hematoxylin and eosin.

The other adrenal was fixed in 10% neutralized formalin for a week and then sectioned, without any embedding medium, on the freezing microtome at 15 μ . Sections including the medulla were prepared in the following ways: (1) stained for one minute with a 1% solution of sudan black B in 70% alcohol, (2) stained by the Schultz cholesterol method, (3) stained by the Ashbel-Seligman ('49) method for ketonic steroids, (4) mounted unstained, and (5) extracted for one-half hour with acetone at room temperature and then mounted. The unstained sections were compared with those that had been extracted with acetone under the polarizing and fluorescence microscopes. These methods as a group are believed to localize the sites of formation of the cortical hormones; they have been found to aid in evaluating the secretory activity of the gland as well (Deane, Shaw and Greep).

Mitotic counts were made on the hematoxylin and eosin preparations of adrenal glands from two rats of each age group. Four sections near the middle of each gland were studied under the oil-immersion objective. All unquestionable mitotic figures in metaphase and anaphase were recorded as to their position in the cortex, i.e., in the zona glomerulosa (including the transitional zone) or in the zona fasciculata. The area of the cortex in these same sections was measured with a planimeter on a $\times 40$ outline made with the aid of the camera lucida. The mitotic ratio was then calculated as the number of mitotic figures per square millimeter of cortex.

RESULTS

No differences, either histological or cytochemical, were detected between the adrenal glands of the male and female rats of the same age. Consequently the descriptions which follow are based on study of the glands from all 4 animals of each age group.

Morphology of the developing adrenal cortex

At birth, the rat's adrenal gland is bounded by a well-defined capsule and contains both cortical and medullary tissue. The medulla is still incompletely organized, however, with the center of the gland consisting of strands of cortical cells intermixed with clumps of medullary cells. This intermixture is best seen in frozen sections stained with a sudan dye, by reason of the fatty inclusions in the cortical cells (fig. 2). During the first week after birth the medulla becomes unified, while the cortical cells located in the central area shrink and gradually disappear (fig. 3). After the first week, cortical cells are only occasionally seen within the medulla (figs. 4 and 5).

From birth, the cortex is separable into two zones, a narrow outer glomerulosa and a broad inner fasciculata. Initially the cells of the glomerulosa are small and possess few vacuoles. They are crowded together without apparent organization (fig. 6). Before the end of a week they acquire numerous fat droplets (fig. 3) and enlarge (fig. 7). Gradually they are organized into the small balls and arcs which characterize the zone in the adult cortex (figs. 8 and 9).

When the cells of the glomerulosa become fatty, a few rows of small spindle-shaped cells which are devoid of fat persist between the glomerulosa and the fasciculata (figs. 3 to 5 and 7 to 9). This zone, which we shall call the transitional zone (Greep and Deane, '49a), was termed the *Grenzschicht* by Engström and the zone of compression by Mitchell.

The fasciculata is composed initially of uniformly large, vacuolated cells which lack apparent organization (fig. 6). Within a week, distinct radially-directed cords develop (fig. 7). They follow a straight course to the border of the medulla (fig. 10) until the end of the second week, when a small *zona reticularis* develops (fig. 11). This reticulate organization of the cords at the border of the medulla becomes more prominent in the older animals (fig. 12). At no time, however, are the staining properties of this zone different from those of

the rest of the cortical cells in hematoxylin and eosin preparations. Moreover, the nuclei in this region resemble those lying more peripherally in the cortex (fig. 8). Even at the end of 6 weeks, the signs of cellular degeneration which often characterize the reticularis in the adult animal have not made their appearance.

TABLE 1

The number of mitotic figures in the capsule and in the cortex of the adrenal gland, the cortical area and the mitotic ratio in sections of adrenals from young rats. Four adrenal sections from each of two rats per age group were studied and the results averaged

AGE OF RATS IN DAYS	NUMBER OF MITOSES PER SECTION				AREA OF CORTEX PER SECTION IN MM ²	MITOTIC RATIO: NUMBER MITOSES PER MM ² CORTEX
	Capsule	Adrenal cortex				
		Glom. ¹	Fasc. ¹	Total		
1	2.0	6.5	1.7	8.2	0.554	14.8
3	4.0	7.0	4.4	11.4	0.578	19.7
5	2.8	4.0	1.0	5.0	0.512	9.8
7	2.2	5.5	8.9	14.4	0.544	26.5
9	2.0	7.8	5.0	12.8	0.616	20.8
11	0.8	6.8	6.6	13.4	0.963	13.9
14	1.5	15.0	25.6	40.6	1.125	35.0
16	2.6	10.1	17.9	28.0	1.548	18.1
22	0.7	9.9	14.8	24.7	2.213	11.1
29	0.4	13.9	4.8	18.7	3.047	6.1
42	0.2	0.5	1.5	2.0	3.245	0.6

¹ Abbreviations: Glom. = zona glomerulosa (plus the transitional zone). Fasc. = zona fasciculata.

Mitotic figures are frequently observed in the glands of rats up to three to 4 weeks of age, but have almost entirely disappeared in the animals 6 weeks old. They occur both in the capsular fibroblasts and in the cortical parenchyma (figs. 6 to 9). In the cortex, divisions are most common in the glomerulosa and outer fasciculata. They occasionally appear at deeper levels, however, especially in animals two weeks of age and older (fig. 8). They have also been observed, though infrequently, within the transitional zone.

The average number of mitotic figures (metaphases and anaphases) occurring in the capsule and in the two principal

zones of the cortex are given in table 1. In the capsule, they are relatively infrequent in the younger rats and virtually disappear after three weeks. In both the glomerulosa and fasciculata cell divisions are numerous in rats up to 4 weeks of age. When the mitotic ratio is calculated as the number of mitoses in the cortex (exclusive of capsule) to the cross-sectional area, peaks in mitotic activity are seen to occur at three days, at 7 days, and again at 14 days. The latter two increments are associated with periods of marked enlargement of the gland, as revealed by the cross-sectional areas listed in the table and also by comparison of the figures 3 to 5 and 10 to 12. Cell multiplication during the first week appears to be offset by the degeneration of cortical cells lying in and around the medulla (compare figs. 2 and 3).

Lipids in the developing adrenal cortex

At the time of birth, the cells of the zona glomerulosa contain only occasional small fat droplets, whereas those of the rest of the cortex are uniformly crowded with small lipid droplets (fig. 2). By 5 days, the glomerulosa has acquired a considerable amount of lipid (fig. 3) and remains fatty thereafter (figs. 4 and 5). After about two weeks, the zona fasciculata develops a gradient of fat content, with more persisting in the outer portion than further inward.

From birth, the lipid droplets in both zones display additional reactions which are characteristic of steroid-producing cells, i.e., the Schultz reaction for cholesterol and related substances (fig. 13), the Ashbel-Seligman reaction for ketonic steroids (fig. 14) and birefringence (figs. 15 and 16). In the youngest animals, the staining reactions are faint and the birefringent crystals exceedingly fine. With progressing age the staining becomes more intense and the crystals coarser. Only in the 6-week-old rats, however, has it been possible to detect acetone-soluble fluorescent materials.

As the medullary tissue coalesces during the first week of life, the cortical cells lying within the medulla and those lo-

cated on its border become more intensely fatty than those lying in the fasciculata proper (fig. 3). These intensely fatty cells gradually diminish in number and after the second week occur only in isolated groups (fig. 4). The droplets in these cells are larger than those found in the rest of the cortex. Initially these droplets give the Schultz test, stain bluish with the Ashbel-Seligman reagents and contain birefringent crystals. By the end of two weeks, however, even though large fat droplets remain in some cells, these "steroid" reactions are negative (figs. 13 to 15).

By 6 weeks lipid droplets have appeared in the cells of the zona reticularis in some of the rats (fig. 5). These droplets are small and display the special reactions (fig. 16).

DISCUSSION

In the adrenal cortex of the rat the zona glomerulosa is distinguishable from the zona fasciculata from the time of birth by reason of its morphological appearance and lipid content. Initially, the outer zone contains little lipid, in contrast to the fasciculata. Within the first week the glomerulosa acquires lipid and becomes separated from the fasciculata by a transitional zone of small, fat-free cells. The description of our material corresponds essentially to that given by Jackson, Engström, and Tobin and Whitehead.

The cytochemical tests we have used demonstrate that, at all ages, the fatty droplets in both the glomerulosa and in the fasciculata display reactions which are characteristic of ketosteroids. Not only do they give the Schultz cholesterol test, as reported by Tobin and Whitehead and Harrison and Cain ('47), but they react with a carbonyl reagent and also contain birefringent crystals. Fluorescence is not detectable until the rat is 6 weeks of age; this reaction, however, is relatively insensitive. At first the droplets are small, the special staining reactions weak and the birefringent crystals fine; gradually the droplets enlarge, stain more darkly and contain coarser crystals. Droplet size and coarseness of the birefringent crystals have been found to be correlated with

secretory rate: small droplets characterize rapid secretion, larger ones a slower release (Deane, Shaw and Greep). The presence of only small droplets of reactive lipid in the cortex at the time of birth suggests that secretion of cortical hormones is relatively rapid at this time. Later, as the droplets and the birefringent particles enlarge, the rate of turnover may be somewhat slower. The emergence of fluorescence at 6 weeks also suggests that some storage is occurring by this age. While we have no physiological evidence for these conclusions, tests on the urine of human infants reveal the presence of glycogenic steroids of cortical origin on the first day after birth (Venning et al., '49). Moreover, Jailer ('49) has shown that pituitary adrenocorticotropic hormone stimulates the adrenal in the 4-day-old rat. Adrenocorticotropin causes the release of glycogenic hormones in the rat, and these steroids appear to be formed by the zona fasciculata (Bergner and Deane, '48). No information is available, so far as we know, on the secretion of salt-regulating hormones by the adrenal cortex of the infant. These hormones are believed to come from the glomerulosa, and their release does not depend on the pituitary (Deane, Shaw and Greep).

No signs of an X zone have been detected in these young rats. The cells which initially lie within the medulla and on its border become increasingly fatty and gradually disappear. The lipid dropets in these cells, however, at first display special reactions suggesting the presence of steroids. The fat which makes its appearance in the X zone of the mouse as it undergoes involution displays none of these reactions (Jones, '49). Moreover, we have been unable to discern any distinctive staining properties or nuclear differences in the juxtamedullary cells which would suggest the existence of a peculiar zone such as that described by Mitchell. We therefore find ourselves in agreement with Tobin and Whitehead that the rat's cortex does not develop an X zone, as was claimed by Howard. We are unable to assess Jackson's suggestion that it is pressure exerted by the growing medulla

which causes the degeneration of the innermost cells of the cortex immediately after birth.

During the first week of life the cross-sectional area of the adrenal cortex does not appear to enlarge, although cell division is moderately frequent. Apparently a balance exists between cell multiplication on the one hand and cell degeneration at the medullary border on the other. During the next three weeks, however, the cortex grows rapidly (table 1). Jackson found that the cortical cells do not enlarge significantly after birth, and our preparations confirm this (plate 2). Mitotic division in cortical cells, however, is frequent during this period, and the mitotic ratio reaches its highest peak at approximately 14 days. The divisions are distributed throughout the cortex, although, as in older rats (Baxter, '46; Mitchell), the majority lie in the glomerulosa and outer fasciculata. Both Engström and Mitchell describe mitosis as absent in the transitional zone, although present both outside and below this region. While we have occasionally seen mitotic figures in the transitional zone, our observations in general confirm theirs. These results lend support to the hypothesis that the normal growth of the cortex involves cell division within the respective zones, not a migration from the glomerulosa inward (Greep and Deane, '49a, b). Moreover, since cell division is relatively rare in the capsule, it may account solely for the growth of that structure as the cortex enlarges, rather than serving as a source of new parenchymal cells.

SUMMARY AND CONCLUSIONS

The adrenal cortex of young male and female rats aged one to 42 days has been studied histologically and cytochemically. No sex differences have been detected in the glands.

From birth the cortex is separable into two major zones, a glomerulosa and a fasciculata. The glomerulosa contains little lipid initially but acquires it during the first week. The fasciculata is uniformly fatty at birth; later a descending gradient develops, with more fat persisting in the outer portion than further inward. The cortical cells lying in and

around the medulla at the time of birth degenerate during the first two weeks of life. Neither these cells nor those which thereafter lie at the medullary border have the appearance of an X zone, contrary to the conclusion of Howard.

The lipid droplets in both the glomerulosa and fasciculata display reactions which are characteristic of ketosteroids. The droplet size is initially small, suggesting that secretion is rapid. Later the droplets enlarge, indicating some storage.

The cortex grows rapidly between 7 and 28 days. The growth depends principally on cell division. Mitotic figures occur in both the glomerulosa and fasciculata, suggesting the absence of cell migration. Mitosis is relatively rare in the capsule.

It is concluded that the functional zonation which characterizes the cortex of the adult rat extends back to birth and that the cells in both the fasciculata and glomerulosa are actively secreting in the infant adrenal.

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PLATE 1

EXPLANATION OF FIGURES

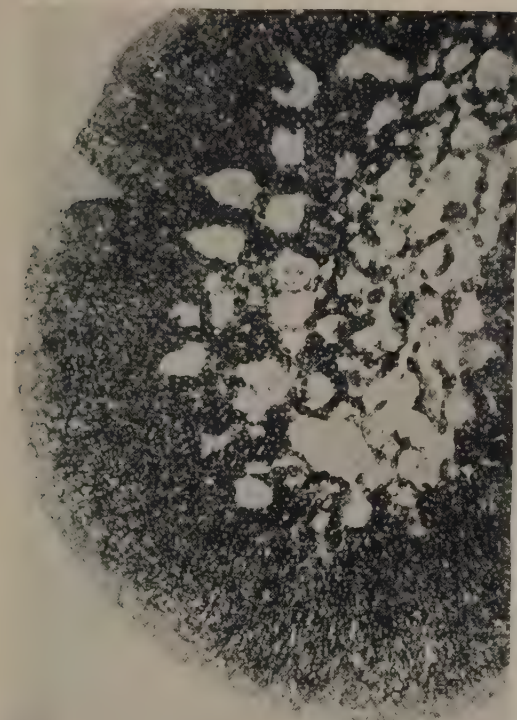
All figures on this plate are from adrenal glands that were fixed in 10% formalin, sectioned at 15μ on the freezing microtome, and stained with sudan black B. Photographed with a blue filter (Wratten H). $\times 95$.

2 Female rat, one day old. The adrenal gland is composed of fatty cortical cells and fat-free medullary cells. In the center of the gland, cortical strands are intermixed with the medullary tissue. The outermost zone of the cortex, the glomerulosa, contains relatively little lipid; the rest of the cortex is uniformly fatty.

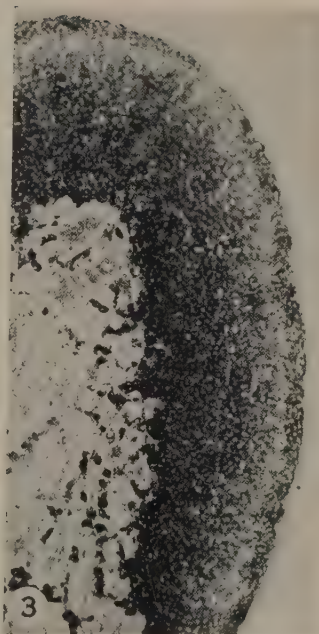
3 Female rat, 5 days old. The medulla is now well defined; only a few cortical cells remain within it. These cells and those immediately adjacent to the medulla are more intensely fatty than the other cortical cells. The glomerulosa contains considerable amounts of lipid and is separated from the fasciculata by a relatively fat-free transitional zone.

4 Female rat, 16 days old. The cortex presents the appearance characterizing the adult gland, with a well-defined glomerulosa, transitional zone and fasciculata. The outer part of the fasciculata contains more lipid than the inner part. A few intensely fatty cells remain at the border of the medulla and within it.

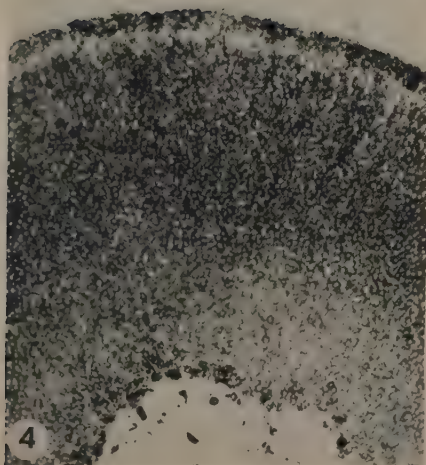
5 Male rat, 42 days old. The cortex has enlarged but otherwise resembles that shown in figure 4. The inner fasciculata contains little fat, while the cells of the reticularis display small amounts.



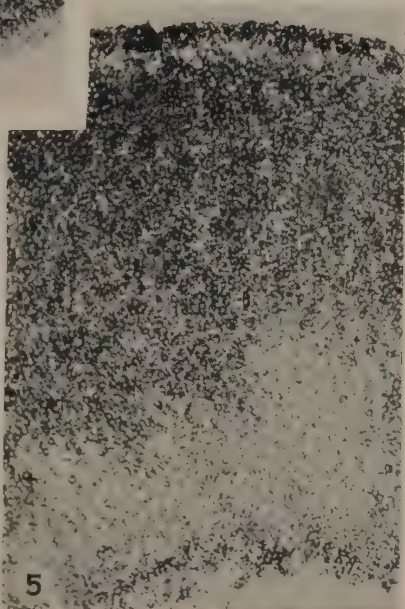
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PLATE 2

EXPLANATION OF FIGURES

All figures on this plate are from adrenal glands that were fixed in Bouin's fluid, sectioned in paraffin at 10μ and stained with Harris' hematoxylin and eosin. Photographed with an orange filter (Wratten E). $\times 480$.

6 Female rat, three days old. The capsule, glomerulosa and outer fasciculata are shown. The cells of the glomerulosa are smaller and more crowded than those in the fasciculata. Mitotic figures are seen in the glomerulosa, the outer fasciculata and the capsule.

7 Female rat, 7 days old. The cells of the glomerulosa are larger than at three days. Mitotic figures occur in the capsule and outer fasciculata.

8 Male rat, 14 days old. The entire cortex is shown, with the edge of the medulla appearing the lower right-hand corner of the picture. Mitotic figures occur in the capsule and throughout the fasciculata. Note particularly the one lying only a few cell layers from the medulla.

9 Male rat, 42 days old. The definitive organization of the adult cortex is illustrated. A pair of telophasic nuclei occurs in the outer fasciculata.

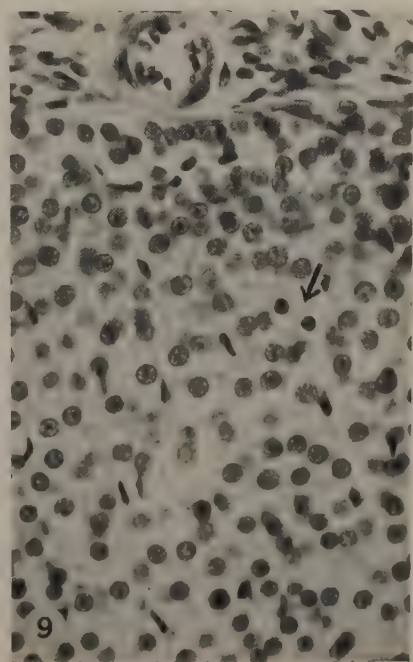
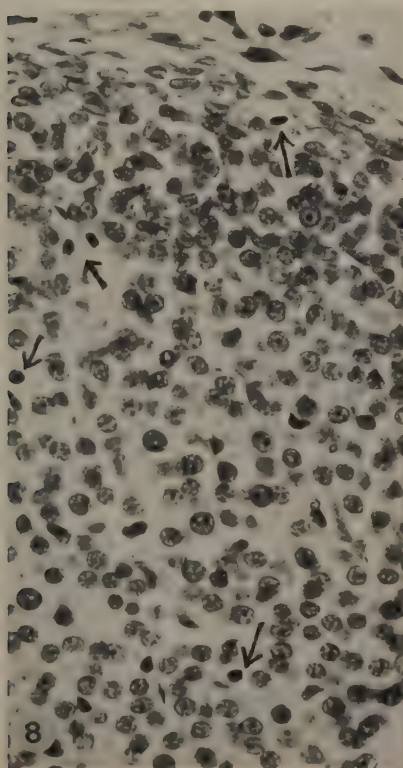
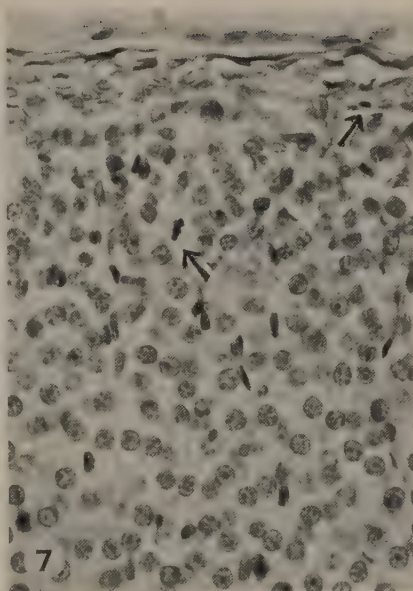
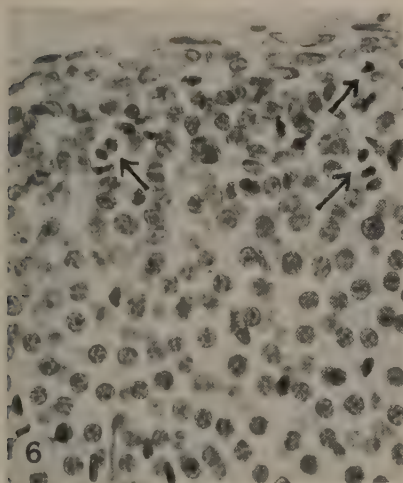


PLATE 3

EXPLANATION OF FIGURES

Figures 10 to 12 are from adrenal glands that were fixed in Bouin's fluid, sectioned at 10μ , stained with hematoxylin and eosin, and photographed with green and yellow filters combined (Wratten B and G). Figures 13 to 16 are of adrenal glands that were fixed in 10% formalin and sectioned on the freezing microtome at 15μ . $\times 95$.

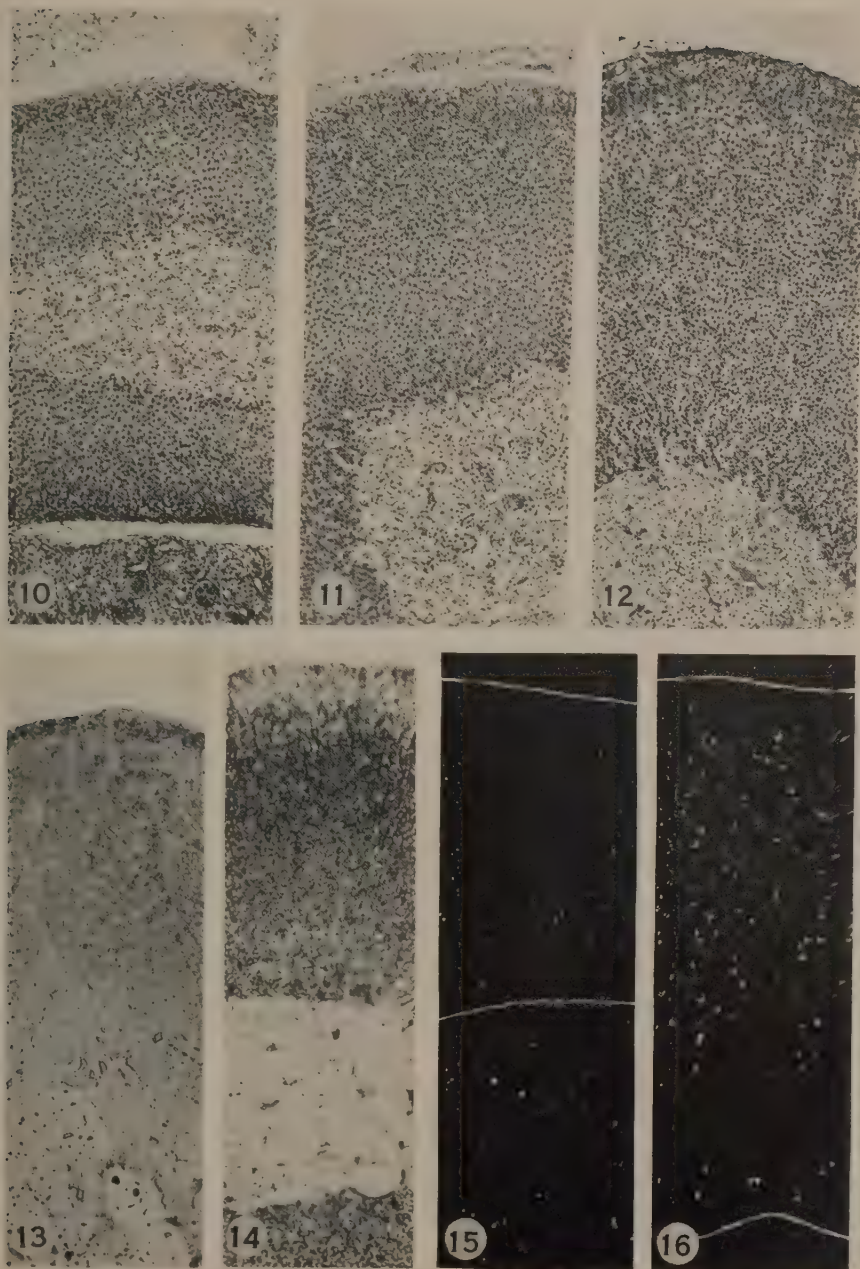
10, 11 and 12 Male rat, 11 days old; female rat, 16 days old; male rat, 42 days old. Compare the width of the cortex at these three ages. By 16 days and more clearly at 42 days, the innermost part of the cortex has acquired a reticulate organization.

13 Female rat, 16 days old. Section stained by the Schultz method for cholesterol. Photographed with a red filter (Wratten F). The droplets in the glomerulosa and outer fasciculata contain material which gives the characteristic blue-green reaction.

14 Male rat, 22 days old. Section stained by the Ashbel-Seligman method for ketonic steroids. Photographed with a green filter (Wratten B). Some of the droplets in the glomerulosa and those in the outer fasciculata stain the positive blue-purple color.

15 Female rat, 16 days old. Untreated section photographed under the polarizing microscope. The outer border of the capsule and the inner border of the cortex have been drawn in. Exceedingly fine birefringent crystals occur throughout the cortex and in the occasional cortical cells lying in the medulla.

16 Male rat, 42 days old. Birefringence picture. The birefringent particles in the glomerulosa are still fine and hence do not show in the photograph. Those in the fasciculata are now mixed in size. Note occasional large crystals in the zona reticularis (compare with fig. 5).



THE MORPHOLOGY OF THE NERVE CELL NUCLEUS, ACCORDING TO SEX¹

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TWELVE FIGURES

INTRODUCTION

Cytologists are accustomed to think of sex differences in nuclear morphology in terms of the complement of chromosomes, as seen during cell division. Such studies are difficult in mammalian cells because of the small size and large number of chromosomes. However, the difference in chromosome content of nerve cells in male and female cats leaves its imprint on the "resting" nuclei. An intranuclear body, which appears morphologically as a small satellite to the large nucleolus, is better developed in female than in male cells. We wish to present further details concerning this interesting sex difference, as an elaboration of a preliminary report by Barr and Bertram ('49).

As a temporary convenience, the term "nucleolar satellite" will be used for the sex-influenced body. It is not to be confused with the satellite which occurs at the end of a nucleolar chromosome in some species. As data accumulate, the authors hope that those better informed than ourselves in the field of cytogenetics will come to our assistance in the matter of terminology.

MATERIALS AND METHODS

Twelve mature cats, 6 males and 6 females, were used. Under Nembutal anesthesia, the tissues were fixed with iso-

¹ This investigation was carried out with the aid of grants from the National Research Council of Canada and the National Cancer Institute of Canada.

tonic 10% formalin by perfusion through the vascular system. Blocks were taken from various regions of the nervous system and embedded in paraffin. Sections cut at $12\ \mu$ and stained with cresyl violet were used for routine study of nuclear structure, and recording the incidence of the nucleolar satellite and its position within the nucleus; $5\ \mu$ sections, also stained with cresyl violet, were prepared for more detailed study and photomicrography. The animals were treated as 6 pairs, each pair consisting of a male and a female. In order to increase the uniformity of staining, sections from the same region from a male and a female cat were mounted on the same slide. In each region, 500 cell sections containing a nucleolus were examined for the presence of a nucleolar satellite. When visible, the position of the satellite was recorded under three general headings — adjacent to the nucleolus, free in the nucleoplasm and adjacent to the nuclear membrane.

In addition to the material from the 12 animals mentioned, sections of nervous tissue from a large number of cats of known sex were examined, though less systematically. These sections from the laboratory collection included material prepared by several techniques commonly used in neurohistology.

OBSERVATIONS

Females. The incidence of visible nucleolar satellites in 12 regions of the nervous system is shown in table 1. In order to simplify the table, mean figures for the 6 female cats are recorded. Detailed anatomical localization was not attempted in this initial survey. For example, "hypothalamus" refers to the general hypothalamic area rather than a specific nucleus.

Considering the various regions together, a nucleolar satellite may be identified in three cell sections out of 4. The mammillary bodies were rather difficult to study, the cells being small and unusually susceptible to technical alterations. The relatively low incidence of visible satellites in this region probably does not reflect the true condition.

It is our impression that each nerve cell in the female cat contains a nucleolar satellite. In cell sections in which a satellite cannot be seen, with the cresyl violet stain, it is probably concealed by the large nucleolus or by deeply staining Nissl bodies lying adjacent to the nuclear membrane.

TABLE 1

Incidence and position of nucleolar satellites (%) in 12 μ sections stained with cresyl violet, each cell section containing a nucleolus. Mean of 6 animals of each sex, 500 cells examined in each area in each animal

REGION OF NERVOUS SYSTEM	SEX	POSITION OF NUCLEOLAR SATELLITE (%)			TOTAL (%)
		Adjacent to nucleolus	Free in nucleoplasm	Adjacent to nuclear membrane	
Motor cortex	♀	69.8	5.3	6.3	81.4
	♂	3.2	0.5	0.1	3.8
Purkinje cells	♀	85.7	1.1	0.5	87.3
	♂	4.6	0.6	0.1	5.3
Lateral geniculate body	♀	74.4	3.6	4.4	82.4
	♂	3.2	1.0	0.0	4.2
Hypothalamus	♀	56.8	7.8	11.7	76.3
	♂	4.4	1.8	0.1	6.3
Mammillary body	♀	27.1	3.7	25.3	56.1
	♂	5.4	0.6	0.2	6.2
Hypoglossal nucleus	♀	58.6	10.0	9.0	77.6
	♂	2.2	0.6	0.3	3.1
Dorsal root ganglion (cervical)	♀	39.5	11.1	17.0	67.6
	♂	2.6	1.0	0.4	4.0
Dorsal horn (cervical)	♀	55.2	7.4	11.6	74.2
	♂	3.3	1.8	0.0	5.1
Dorsal horn (lumbosacral)	♀	59.4	7.4	8.1	74.9
	♂	2.6	1.7	0.0	4.3
Ventral horn (cervical)	♀	48.5	13.9	11.6	74.0
	♂	2.7	1.8	0.2	4.7
Ventral horn (lumbosacral)	♀	56.9	12.8	9.0	78.7
	♂	2.3	2.0	0.1	4.4
Stellate ganglion	♀	54.0	9.9	13.6	77.5
	♂	1.5	0.5	0.1	2.1

The position of the nucleolar satellite is also shown in table 1. The satellite is situated adjacent to the nucleolus most frequently (figs. 1, 3, 5, 7 and 10); less often the satellite lies free in the nucleoplasm (fig. 8) or adjacent to the nuclear membrane (fig. 11). The morphological relationship between nucleolus and satellite is especially close in Purkinje cells of the cerebellum. The position of the nucleolar satellite may be altered by experimental conditions which affect the metabolism of the neuron (Barr and Bertram, '49). We are inclined to think that the variations in the position of the satellite under normal conditions may also rest on a functional or metabolic basis. The position pattern of the nucleolar satellite is a further item for consideration in the descriptive morphology of different types of nerve cells.

The nucleolar satellite of the female is spherical or irregular in shape. When adjacent to the nucleolus the satellite may be flattened on its nucleolar side. This is commonly seen in Purkinje cells. A narrow interval between the nucleolus and adjacent satellite is often visible, and the surface of the nucleolus may be flattened (fig. 7) or show a shallow concavity at this point. When the satellite lies against the nuclear membrane it is typically flattened on the membrane side, presenting a triangular or semicircular outline in profile view (fig. 11). Since the satellite changes in shape to conform to adjacent structures, yet retains its individuality as a discrete body, it is probably semisolid in consistency. When the satellite is free of the nucleolus no strands are seen connecting the two structures.

The nucleolar satellite of the female is approximately $1\ \mu$ in diameter and, unlike the nucleolus, its volume is remarkably constant from one cell type to another. The nucleolus and satellite were measured in 50 small cells of the dorsal horn and in 50 large cells of the ventral horn of the spinal cord. The average measurements, expressed as volumes, are as follows — dorsal horn cells: nucleolus, $10.5\ \mu^3$, satellite $0.5\ \mu^3$; ventral horn cells: nucleolus, $43.4\ \mu^3$, satellite, $0.5\ \mu^3$.

The position of the satellite appears to bear no relation to the polarity of the neuron. This point was investigated systematically in the hypoglossal nucleus. The axons arise predominantly from the ventromedial aspect of the cell bodies; the satellites have a random spatial orientation.

The satellite, like the nucleolus, stains readily with basic dyes. However, its intensity of staining is variable. The satellite may stain with about the same intensity as the nucleolus, or it may have an increased or decreased affinity for cresyl violet, compared with the nucleolus. Slight variations in staining technic may be responsible, but quantitative or qualitative differences in the chemical composition of the satellite of different cells cannot be excluded. Cells in which the satellite stains more deeply than the nucleolus are most favorable for study. The satellite may then be identified, by careful focusing, even though it is located in the same optical axis as the nucleolus or at the nuclear membrane close to perinuclear Nissl material.

While vacuoles are seen frequently in the nucleolus, no details of internal structure are seen in the nucleolar satellite.

Males. The small size of the nucleolar satellite in male nerve cells prevents satisfactory examination with a standard light microscope. In a small proportion of male neurons a discrete particle is visible, though smaller than the satellite of female cells (table 1, average data for 6 male cats). In many cells the satellite may be just above the limit of resolution but impossible to distinguish from a particle of unrelated chromatin. Because of these difficulties, the figures shown in table 1 are only approximate. They serve the purpose, however, of emphasizing the clear difference in nuclear structure, according to sex. Typical male neurons are illustrated in figures 2, 4, 6, 9 and 12.

The impression that all male neurons contain a nucleolar satellite, though usually too small for accurate identification, is confirmed by certain experimental observations (to be published). Extensive depletion of the Nissl material of hypo-

glossal nerve cells follows prolonged antidromic stimulation. During the recovery phase, in male cells, nucleolar satellites become clearly visible in as high as 38% of cells, although normally the satellite can be identified in less than 5% of cells. Apparently the satellite enlarges during the altered metabolic state of the cell so that a considerable number come to fall within the limits of optical resolution with an oil immersion objective.

Although satellite size differs according to sex, routine examination of the sections conveys the impression that nucleolar volume is the same in males and females. This impression was confirmed by careful measurements in the lateral geniculate body. The average nucleolar volume varies slightly in different animals of the same sex. However, when the values for all of the males are averaged and compared with mean values for all of the females, identical figures result.

Stains other than cresyl violet. The observations which follow apply to nerve cells of female cats. The frequency of visible nucleolar satellites in male neurons remains consistently low regardless of the staining procedure.

(a) *Thionin.* This stain, applied to celloidon sections, is especially useful because of its metachromatic properties. The Nissl material and nucleolus stain violet; the nucleolar satellite stains blue. This differential staining facilitates identification of the satellite even when it lies in the same optical axis as the nucleolus or at the periphery of the nucleus and close to masses of Nissl material. More accurate information concerning the incidence and position pattern of the satellite can be obtained with thionin than with cresyl violet. Cells of the lateral geniculate body in 6 female cats were examined systematically after celloidon embedding and staining with thionin (14 μ sections). The mean figures are as follows — adjacent to nucleolus, 77.2%; free in nucleoplasm, 2.1%; adjacent to nuclear membrane, 16.7%; total, 96.0%.

(b) *Feulgen and methyl green-pyronin technics.* These methods are valuable for their cytochemical properties. The nucleolar satellite is consistently Feulgen-positive, while the

nucleolus and Nissl material are Feulgen-negative. With methyl green-pyronin the satellite stains green; the nucleolus and Nissl material stain red. These observations, combined with data on the cytochemistry of the neuron now in the literature (Hydén, '47), indicate that the nucleolar satellite contains desoxyribose nucleic acid, the nucleolus and Nissl material ribose nucleic acid.

(c) *Silver methods and iron hematoxylin.* The nucleolar satellite is well stained by Bodian's protargol method, various modifications of Cajal's reduced silver nitrate method and by iron hematoxylin. For the present purpose, the latter methods have the disadvantage of staining considerable intranuclear material in addition to the nucleolus and its satellite.

Human nerve cells. It is our intention to restrict this report almost exclusively to a description of the nerve cell nucleus of the cat. However, in the section to follow, there will be occasion to refer to the nucleolar chromosomes of man. Certain general statements on nuclear morphology in the human neuron are, therefore, required. Detailed observations on human material will be reported later.

Human sympathetic ganglion cells show the same consistent sex difference as occurs throughout the nervous system of the cat. In female cells, the satellite is situated frequently at the nuclear membrane where it may be flattened in the form of an exceedingly thin disc. The nucleolus is often eccentric in the same direction, the satellite lying between nucleolus and nuclear membrane and touching both. Nucleolar satellites are seldom encountered in male sympathetic ganglion cells and, when visible, are very small.

Human material, however, shows interesting variations in nuclear morphology according to the cell type. There is very little sex difference in Purkinje cells. Nucleolar satellites are visible with about the same frequency in male and female Purkinje cells but are, on the whole, a little smaller in the male. The difference is insufficient to permit identification of the sex of the individual from whom a section of cerebellar cortex was obtained. Pyramidal cells of the cerebral cortex

(area 4) lie between the extremes presented by sympathetic ganglion and Purkinje cells. Our preliminary study of human material suggests that nuclear morphology, from the standpoint of sex difference, is similar in the human and the cat but that the nucleolar satellite of the male human enlarges in certain types of neurons, possibly as a result of functional demands made upon it.

DISCUSSION

The basis for a sex difference in the morphology of the resting nucleus is a question of compelling interest. In the absence of direct evidence, the brief discussion which follows must be regarded as exploratory and subject to extensive modification as more facts become available.

According to de Winiwarter ('20), the chromosome complement for the cat is $34 + XX$ for the female and $34 + XO$ for the male. Our reasoning is based on the assumption that the difference in number of X chromosomes is the primary factor which determines the distinction between a female and male resting nucleus.

Insects and plants are the main source of data concerning chromosome morphology. In these forms the X chromosome is often the nucleolar chromosome (Gates, '42). Accordingly, Barr and Bertram ('49) suggested that the nucleolar satellite may be derived from that portion of the nucleolar chromosome known as the nucleolar organizer, the female having a double complement of this material, compared with the male. This interpretation now appears unlikely.

The nucleolus and the nucleolar satellite may not be as intimately related as the morphological appearance of the resting nucleus suggests. Schultz and St. Lawrence ('49), in an important paper, have shown that the nucleolar chromosome of man is an autosome. The formation of the nucleolus in man should not be influenced by the sex of the individual, since it arises from a pair of autosomes in both males and females. The equal nucleolar volume in male and female cats could be regarded as a point in favor of a similar origin of the nucleolus in the cat. Schultz and St. Lawrence have also

recorded the significant observation that, in man, "a nucleolus is formed by the X chromosome, independent of that formed by the nucleolar chromosome." It is possible that a similar phenomenon occurs in the cat, the body formed by the X chromosome becoming the nucleolar satellite of the resting nucleus. If this is the case, the body formed by the single X chromosome of the male is so small that it is seen with difficulty in the resting nucleus. In the female, however, two small masses of X chromosome origin would be available to fuse and form a nucleolar satellite of appreciable size. It is to be hoped that workers familiar with the technics of cytogenetics will be able to provide data for the cat and other mammals similar to that recorded by Schultz and St. Lawrence for man.

The knowledge that a nuclear component differs in size according to sex may be expected to serve as a stimulus to inquiry into its function in cell metabolism. Experiments in this laboratory clearly indicate that the nucleolar satellites of male and female neurons enlarge during the recovery of a cell from chromatolysis induced by prolonged antidromic stimulation (Bertram et al., '50). There is a concurrent enlargement and increased vacuolation of the nucleolus. According to Hydén ('47), the nucleolus participates in the synthesis of ribose nucleoproteins of Nissl material. The enlargement of the nucleolar satellite during accelerated formation of Nissl material suggests that the satellite, as well as the nucleolus, may be concerned with nucleoprotein synthesis. However that may be, it is interesting to speculate that some aspect of nerve cell metabolism may eventually prove to be different quantitatively in the two sexes, in favor of the female.

SUMMARY

1. A difference in the morphology of the nerve cell nucleus of the cat, according to sex, is described. In the female, a body about 1μ in diameter appears as a small satellite to the large nucleolus in all types of nerve cells examined. In the male, a nucleolar satellite is seldom seen distinctly. There is evidence

that male nerve cells contain a nucleolar satellite so small that it lies at the limit of resolution with standard optical equipment.

2. The results of staining with Feulgen and methyl green-pyronin methods indicate that the nucleolar satellite contains desoxyribose nucleic acid, unlike the nucleolus which contains ribose nucleic acid.

3. On the basis of indirect evidence, it is suggested that the nucleolar satellite is a product of the X chromosome. From the cytogenetic point of view, the satellite may be less intimately related to the nucleolus than the morphological appearance of the resting nucleus would suggest.

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PLATES

All illustrations are of nerve cells of the cat, prepared from 5μ sections stained with cresyl violet, magnification $\times 1200$.

PLATE 1

EXPLANATION OF FIGURES

1 Purkinje cell, female. The well defined satellite body, typical of female cells, is situated adjacent to the nucleolus. The morphological relation between the nucleolus and its satellite is especially close in Purkinje cells.

2 Purkinje cell, male. No nucleolar satellite is visible.

3 Motor neuron of the spinal cord, female, with typical satellite located adjacent to the nucleolus.

4 Motor neuron of the spinal cord, male. A nucleolar satellite is not visible.

5 Stellate ganglion cell, female, showing the characteristic female nuclear morphology.

6 Stellate ganglion cell, male, lacking a visible nucleolar satellite.

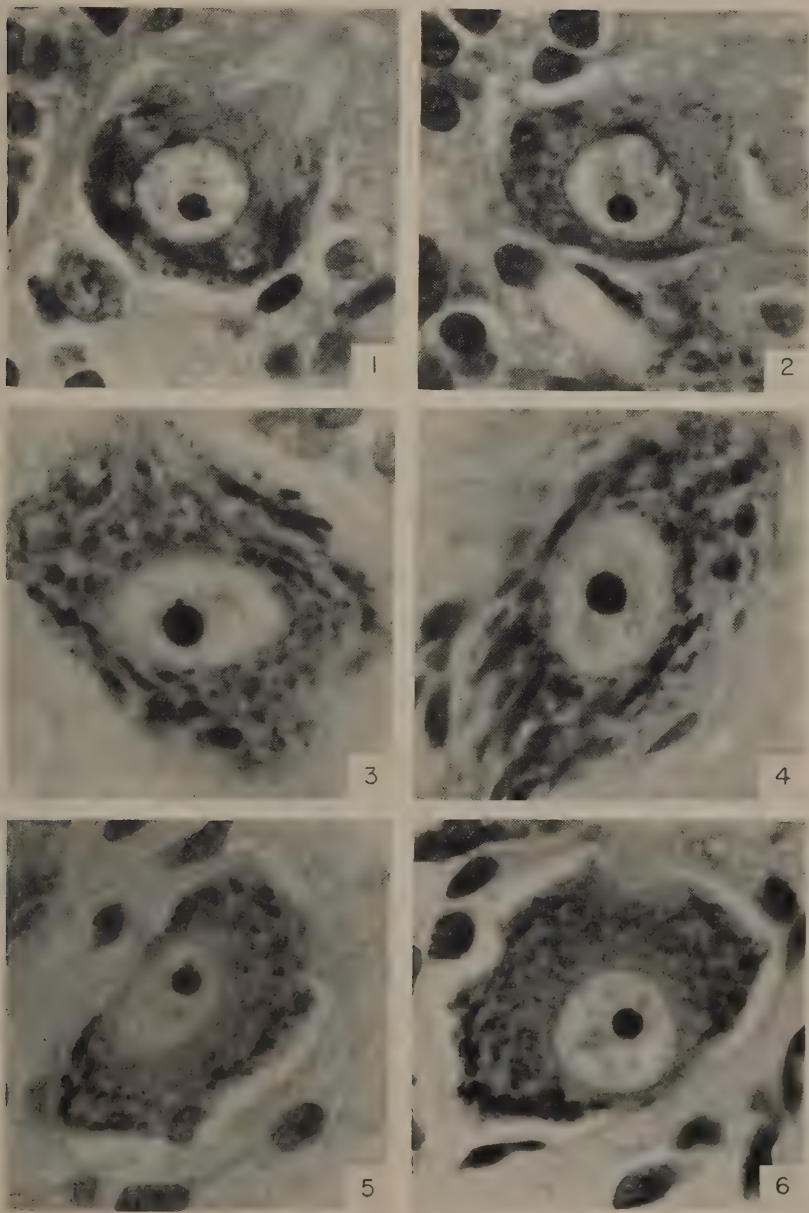
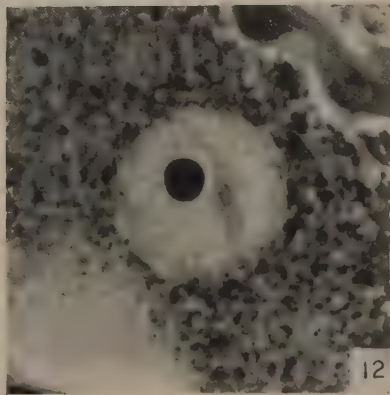
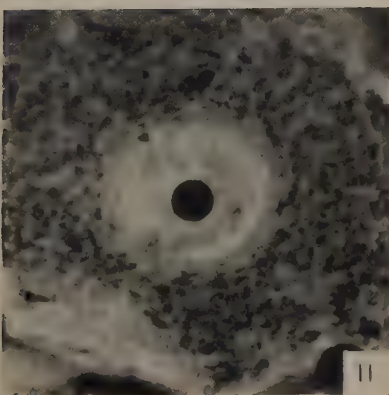
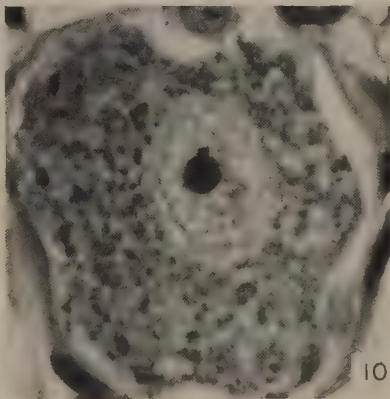
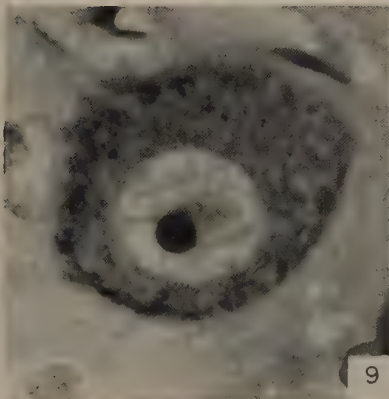
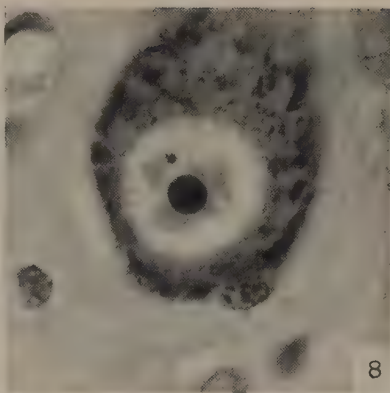
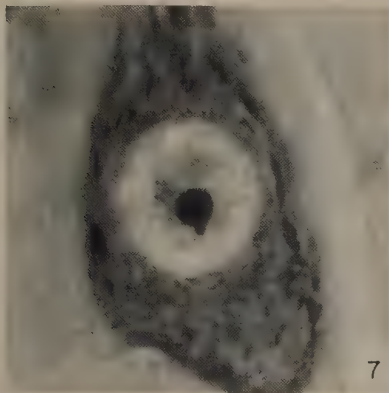


PLATE 2

EXPLANATION OF FIGURES

- 7 Betz cell of motor cortex, female, showing a satellite adjacent to the nucleolus.
- 8 Betz cell of motor cortex, female, to illustrate a nucleolar satellite free in the nucleoplasm.
- 9 Betz cell of motor cortex, male, with no visible nucleolar satellite.
- 10 Dorsal root ganglion cell, female, illustrating typical female nuclear morphology.
- 11 Dorsal root ganglion cell, female. The nucleolar satellite lies against the nuclear membrane.
- 12 Dorsal root ganglion cell, male. There is no visible nucleolar satellite.



RESPONSE BY THE RAT THYRO-PARATHY- ROIDECTOMIZED AT BIRTH TO GROWTH HORMONE AND TO THYROXIN GIVEN SEPARATELY OR IN COMBINATION ¹

III. SKELETAL CHANGES: TIBIA, METACARPAL, AND CAUDAL VERTEBRAE

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THIRTY FIGURES

Two recent reports have dealt with the response of rats thyroidectomized at birth to administration of pituitary growth hormone and thyroxin. The body growth and organ changes were described by Scow et al., '49. Although such rats made substantial gains in body weight and length when treated with pure growth hormone, they remained immature, as did their uninjected thyroidectomized controls, judged both from external appearances and from roentgenograms of the skeleton. On the other hand, when treated with thyroxin, the animals not only grew markedly, but also advanced in maturity, somatic and skeletal. A second report by Koneff et al., '49, demonstrated that the administration of thyroxin repaired the pituitary cytologic deficiencies which had attended

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early thyroidectomy. In particular, the eosinophils (the putative source of growth hormone) were restored to normal. As anticipated, administration of growth hormone did not correct the pituitary pathology. It thus appeared that the excellent balance between growth and maturation shown by the thyroxin-treated rats was due as much to the re-establishment of an endogenous supply of growth hormone as to the direct effect of thyroxin itself. Thyroidectomized rats injected with both of these hormones made gains comparable to, but not substantially in excess of, such gains made by rats injected with thyroxin alone. This suggested that the optimum had been achieved by the thyroxin injections and that additional, exogenous supplies of growth hormone did not appreciably increase the growth rate of these already rapidly growing rats.

It is the purpose of this report, the third in the series, to describe the histologic differences seen in representative skeletal structures of these rats. Special attention was directed in this study to evidences of skeletal maturity, particularly to presence and extent of development of ossification centers. The objective was in part to correlate histologic structure with the roentgenographic diagnoses of "bone age." It was also of importance to seek evidence of imbalance in endochondral ossification which would account for skeletal growth without differentiation in the growth-hormone injected rats, as contrasted with the balanced growth and differentiation in those injected with thyroxin.

The conditions of the experiment have been described in detail in the first report of this series. Briefly, they were as follows: Approximately 300 rats of the Long-Evans strain were thyro-parathyroidectomized at birth. Of these, 60 were selected when 30 days of age as being presumptively completely thyroidless. The basis for this choice was evidence of marked delay in development—pellage, ears, external genitalia, and skeleton. The last seemed most critical, and no rats were included whose roentgenographic "bone age" exceeded 15 days at a chronologic age of 30 days. The 60 rats

thus chosen were divided into 4 groups of 15 rats each. The first group received pure anterior hypophyseal growth hormone, 0.5 mg daily, injected intraperitoneally, for 30 days. Each dose contained 25 rat units of growth hormone, as assayed in hypophysectomized immature female rats (Li, Evans and Simpson, '45). The second group received crystalline thyroxin (Squibb), 2.5 μ g daily, injected subcutaneously for a like period. A third group received both hormones in the dosage specified. The 4th group was retained as uninjected thyroidectomized controls. Two groups of normal rats were sacrificed as controls. One group (20 rats) was 61-64 days of age at autopsy, to correspond in *chronologic age* to the experimental animals at the time of autopsy. The other group (12 rats) was 18 days of age at autopsy; these rats were designated *bone age* controls, since they corresponded closely in size and skeletal immaturity to the uninjected thyroidectomized rats autopsied at 60 days of age.

At autopsy the completeness of thyroidectomy was verified by the radioautographic technique of Reinhardt ('42). After rejection of those showing possibly incomplete thyroidectomy, there remained for study 6 rats injected with growth hormone, 11 with thyroxin, 10 with both hormones, and 6 uninjected thyroidectomized controls. Both sexes were represented in every group.³

After formal fixation and roentgenography of the carcasses in an extended position, representative portions of the skeleton were removed and prepared for histological study. These included the proximal epiphysis of the tibia, the third meta-

³ The major attention of the present study is to the findings on the females, since in this sex more extensive histological skeletal data have been accumulated for normal standards. Males are not illustrated, and are described only when they differed markedly from the females in skeletal structure. Sex differences are probably unimportant in the uninjected thyroidectomized controls and the growth hormone-injected rats, judged from the immaturity of their external and internal genitalia. However, marked genital development and evidences of function were seen in the rats receiving thyroxin, as a consequence of pituitary repair and resumption of gonadotrophin output, and sex distinction is therefore necessary.

carpal, and the 3rd, 8th, and 13th caudal vertebrae. The mandibular condyle, the molar teeth, and the upper central incisors are being described in a subsequent report.

RESULTS

Tibia

The proximal epiphysis of the tibia, its cartilage plate, and the neighboring portion of the tibial diaphysis were examined to determine their size, degree of development, and endochondral ossification activity.

The bone structure of the normal controls, both 18-day-old bone-age controls and 61-64-day-old autopsy controls is shown in figures 1 and 2. The differences in the expansion of the epiphyseal ossification center at these two ages afford a basis for estimating the maturity attained by this bone in the various experimental groups. In the 18-day-old rats the ossification center occupied 26% of the cartilaginous epiphysis, while in the 61-64-day-old rats, it had expanded to become 71% of the epiphysis.⁴

In the thyroidectomized controls (fig. 3) the epiphyseal ossification center was small and had expanded only slightly into the cartilaginous epiphysis. In per cent of total epiphyseal area (40%) it only slightly exceeded the tibia of the young bone-age controls, lending confirmation to the 18-day-bone age diagnosed roentgenographically in these rats. However, the thyroidectomized rats showed a narrower epiphyseal cartilage plate than the 18-day-old normal rats.

The cells were smaller and the matrix more abundant. There was evidence of bone formation on the entire periphery of the epiphyseal ossification center but there appeared to be little erosion of the surrounding cartilage. Next the diaphyseal marrow junction the cartilage cells were enlarged but not vacuolated. The bony trabeculae were sparse and short. The marrow was cellular and its sinusoids so patent that it was liver-like. The cortical bone of the diaphysis was extremely thin.

⁴ The areas occupied by the cartilaginous epiphysis and the ossification centers within them were traced and measured with a planimeter.

In the growth hormone-injected rats (fig. 4) the tibia itself was substantially larger but the epiphyseal ossification center did not expand, and remained of the size of the 18-day-old normal rats. It occupied 25% of the cartilaginous epiphysis. This added confirmation to the conclusion, based on roentgenograms, that the administration of growth hormone to these thyroidectomized rats did not advance the bone age.

At the periphery of the ossification center marrow tufts were present. The entire cartilaginous part of the epiphysis was proliferating. The epiphyseal cartilage plate was more clearly divided into zones than was the thyroidectomized control and showed clearly the delimitation of the future disc. In particular the cells next the diaphyseal marrow junction were enlarged, vacuolated, and subject to active erosion. The marrow cavity was filled with delicate bony trabeculae. The cortical bone was somewhat thicker than in the controls and was very vascular.

In the rats injected with thyroxin (fig. 5) the tibias were bigger than in the preceding groups. The development of the epiphyseal ossification center was complete, and bone sealed its marrow from the surrounding cartilage. The proportion of the epiphysis occupied by this tissue (55%) approached that in the 61-64-day-old normal rats. A true epiphyseal plate had been established.

The plate was very cellular, the majority of chondrocytes being in columns. At the marrow junction there was active erosion and innumerable delicate bony trabeculae extended far into the marrow. They remained parallel even to the secondary spongiosa. The cortical bone of the diaphysis was substantially thicker than in the controls or growth hormone treated rats. The marrow was cellular.

In the rats treated with both growth hormone and thyroxin (fig. 6) the bones were no bigger than the thyroxin-treated group, and resembled them in differentiation. Bone and marrow constituted 68% of the epiphyseal area. They were scarcely distinguishable from normal controls of the same chronologic age except for a wider epiphyseal cartilage plate. This supported the bone-age diagnosis of marked advance in skeletal maturity during treatment with thyroxin.

Metacarpal

Figures 7-12 show that the response of the metacarpal was essentially the same as that of the tibia. In particular figure 9 shows the retarded development of the thyroidectomized controls. Figure 10 shows the excessive cartilage formation resulting from growth hormone injections. Figures 11 and 12 show well the aging which took place when the rats were injected with thyroxin, with or without growth hormone. The cellularity of the marrow in these last two groups was noteworthy.

Caudal vertebrae

The 3rd, 8th, and 13th caudal vertebrae were studied histologically for two distinct purposes. First, information was sought as to whether these representatives of the axial skeleton responded to the various experimental regimens in the same fashion as did appendicular bones. Second, since the degree of development of the caudal epiphyseal ossification centers, as shown in roentgenograms, had been one of the critical factors in diagnosing the skeletal age attained by rats of the different groups, it was especially important here to attempt histological correlations with the roentgenographic findings.

The histologic examination showed that the caudal vertebrae had responded in a manner essentially similar to that described for the tibia and metacarpal. The normal standards for the different vertebral levels in 18-day-old and 61-64-day-old controls are shown in figures 13-15 and 16-18, respectively. The vertebrae of the thyroidectomized controls (figs. 19-21) corresponded in size to those of the 18-day-old "bone-age" controls but were less mature. Although the diaphyses were osseous, the epiphyses were predominantly cartilaginous. Only the cartilage basophilia and vacuolation which adumbrates the appearance of an ossification center could be found. The growth-hormone injected thyroidectomized rats had caudal vertebrae substantially larger, though they remained immature as in the thyroidectomized controls (figs.

22-24). The orderly arrangement of cartilage cells was a conspicuous feature. The rats injected with thyroxin, whether alone (figs. 25-27) or in combination with growth hormone (figs. 28-30) had caudal vertebrae which in size and differentiation approached those of the normal 61-64-day-old controls. The secondary epiphyseal ossification centers were fully expanded and osseous. They were separated from the diaphysis by cartilage plates whose structure indicated active endochondral osteogenesis.

Efforts at correlating histological and roentgenographic findings in the caudal vertebrae were especially instructive in determining the interpretations that could be made from the roentgenographic findings. The discrepancies were not marked enough to invalidate the roentgenographic technique of skeletal age determinations. However, it was not always possible to confirm histologically the roentgenographic diagnosis of the initial establishment of an ossification center. Although radio-opaque areas suggesting small epiphyseal ossification centers were found, the histologic studies often showed only cartilage basophilia (presumptive calcification). Some histological sections showed small epiphyseal ossification centers which did not appear in the roentgenograms. For example, although in 18-day-old normal rats epiphyseal ossification centers were found histologically at both ends of the 8th and 13th caudal vertebrae, no corresponding radio-opaque areas could be detected. Roentgenographic diagnoses of "fusion" of epiphyses with diaphyses of caudal vertebrae were not sustained, since intact cartilage plates were always found on histological examination. However, such plates were often so narrow and lacking in flatness that there was no straight course through which the roentgen rays could pass to cast the image of the plate. Thus, although differences in the 3rd, 8th, and 13th caudal vertebrae had been anticipated on the basis of the roentgenographic findings, they were not sustained by histological study, as shown by the photomicrographs of these three levels in the various groups.

SUMMARY

The skeletal development in rats thyroidectomized at birth and injected from 30 to 60 days of age with growth hormone, thyroxin, or the two combined has been studied histologically at the proximal epiphysis of the tibia, the third metacarpal, and the 3rd, 8th, and 13th caudal vertebrae. Histologic analysis of growth and differentiation was compared with roentgenographic analysis of these animals. There was clear confirmation that the thyroidectomy markedly retarded growth and differentiation, that growth hormone stimulated growth without differentiation, and that thyroxin stimulated both growth and differentiation. No augmentation of either of these effects was seen when the two hormones were administered together. Although the histologic findings generally confirmed the roentgenographic diagnoses, some discrepancies were found. The roentgenograms did not always show the beginnings of secondary ossification centers, nor in them could bony centers be distinguished from calcified cartilage. Also, roentgenographic diagnoses of epiphyseal "fusion" of caudal vertebrae were not sustained histologically. These discrepancies, however, were not so marked as to invalidate the roentgenographic technique of skeletal age determinations.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of central sagittal sections of the proximal epiphyseal region of the tibia of rats. H. and E. stain. $\times 10$.

1 Normal "bone-age" control, 18 days of age at autopsy. Spec. 9285, Pl. B482.

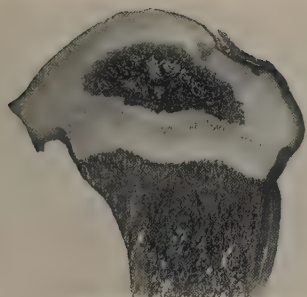
2 Normal control, 63 days of age at autopsy. Spec. 9305, Pl. B483.

3 Thyroidectomized control, 62 days of age at autopsy; thyroidectomized at one day of age. Spec. 9251, Pl. B484.

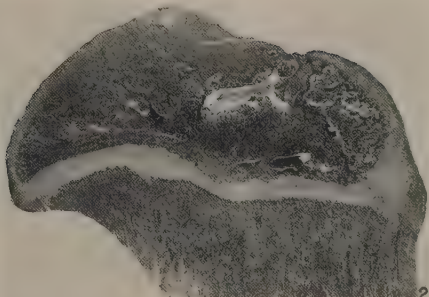
4 Growth hormone, 0.5 mg daily for 30 days, beginning at 32 days of age, thyroidectomized at one day of age. Spec. 9252, Pl. B485.

5 Thyroxin, 0.0025 mg daily for 30 days, beginning at 32 days of age; thyroidectomized at one day of age. Spec. 9259, Pl. B490.

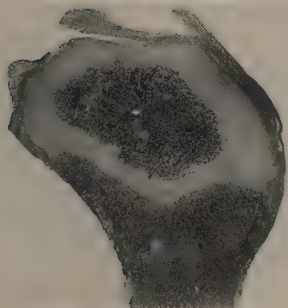
6 Growth hormone and thyroxin, each given as above, beginning at 33 days of age; thyroidectomized at one day of age. Spec. 9262, Pl. B493.



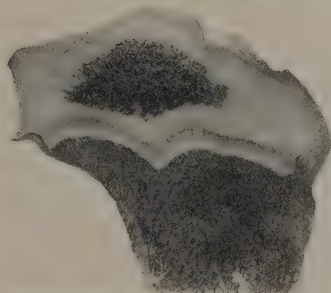
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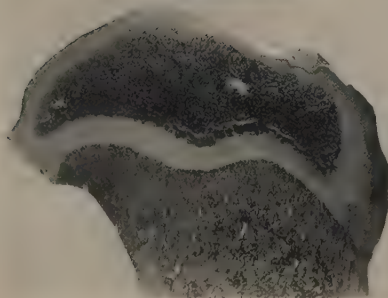
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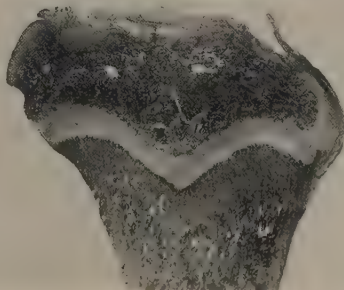
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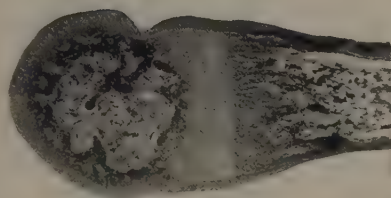
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PLATE 2

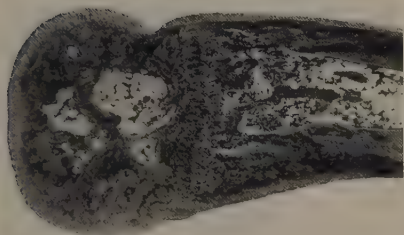
EXPLANATION OF FIGURES

Photomicrographs of the epiphyseal region of the third metacarpal of rats. Median sagittal sections, H. and E. $\times 24$.

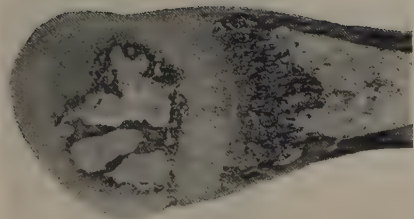
- 7 Normal bone-age control, 18 days of age at autopsy. Spec. 9283, Pl. B531.
- 8 Normal control, 61 days of age at autopsy. Spec. 9302, Pl. B532.
- 9 Thyroidectomized control, 62 days of age at autopsy, thyroidectomized at one day of age. Spec. 9251, Pl. B533.
- 10 Growth hormone, 0.5 mg daily for 30 days, beginning at 33 days of age; thyroidectomized at one day of age. Spec. 9330, Pl. B534.
- 11 Thyroxin, 0.0025 mg daily for 30 days, beginning at 31 days of age, thyroidectomized at one day of age. Spec. 9256, Pl. B535.
- 12 Growth hormone and thyroxin, each given as above, beginning at 34 days of age, thyroidectomized at one day of age. Spec. 9353, Pl. B536.



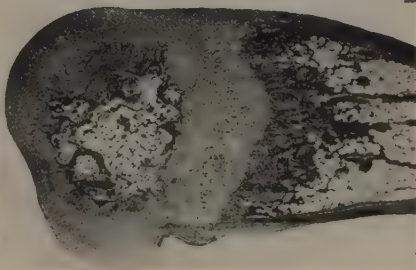
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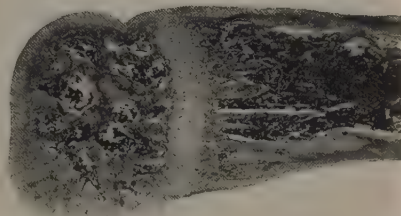
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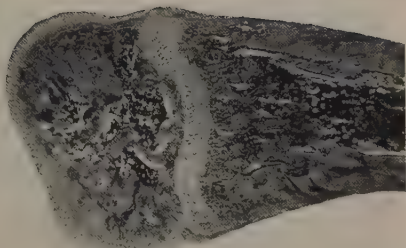
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PLATE 3

EXPLANATION OF FIGURES

Photomicrographs of the epiphyseal region of the 3rd, 8th and 13th caudal vertebrae of normal rats. Median sagittal sections, H. and E. $\times 17$.

Normal bone age control, 18 days of age at autopsy. Spec. 9284.

- 13 Third caudal vertebra. Pl. B555.
- 14 Eighth caudal vertebra. Pl. B556.
- 15 Thirteenth caudal vertebra. Pl. B557.

Normal control 63 days of age at autopsy. Spec. 9295.

- 16 Third caudal vertebra. Pl. B558.
- 17 Eighth caudal vertebra. Pl. B559.
- 18 Thirteenth caudal vertebra. Pl. B560.

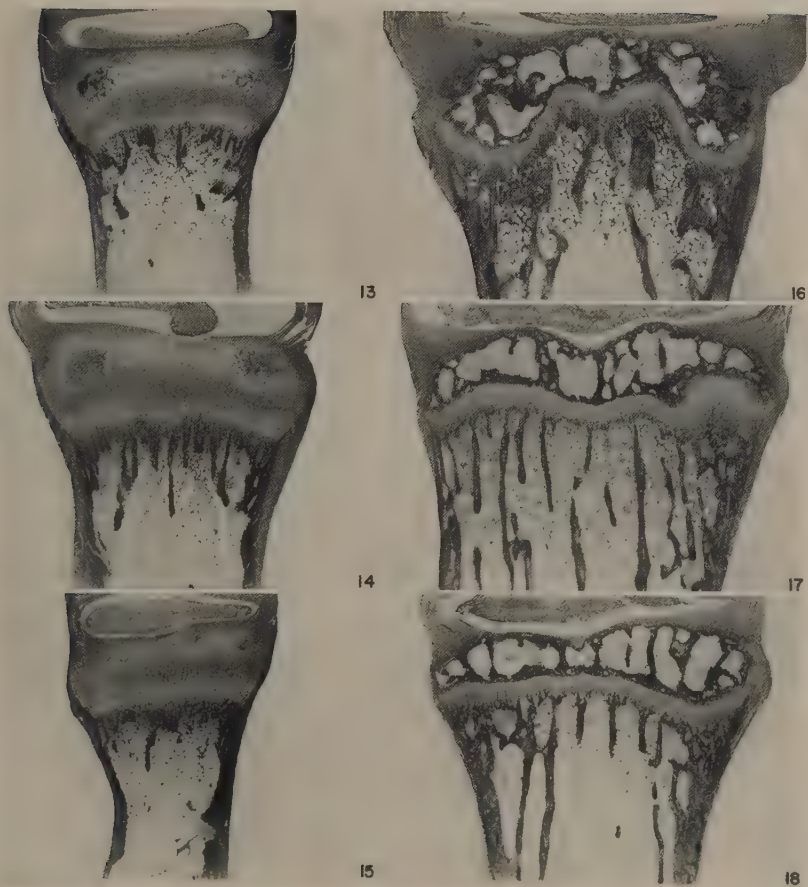


PLATE 4

EXPLANATION OF FIGURES

Photomicrographs of the epiphyseal region of the 3rd, 8th and 13th caudal vertebrae of rats thyroidectomized at one day of age. Median sagittal sections, H. and E. $\times 17$.

Thyroidectomized control, 62 days of age at autopsy. Spec. 9251.

19 Third caudal vertebra. Pl. B561.

20 Eighth caudal vertebra. Pl. B562.

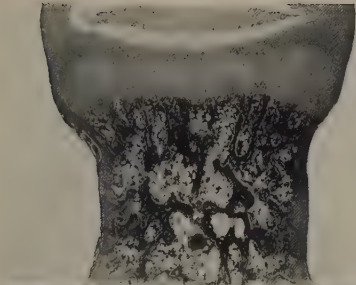
21 Thirteenth caudal vertebra. Pl. B563.

Growth hormone, 0.5 mg daily for 30 days, beginning at 32 days of age. Spec. 9252.

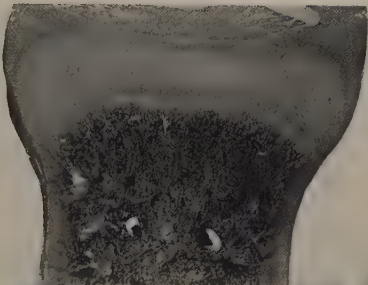
22 Third caudal vertebra. Pl. B564.

23 Eighth caudal vertebra. Pl. B565.

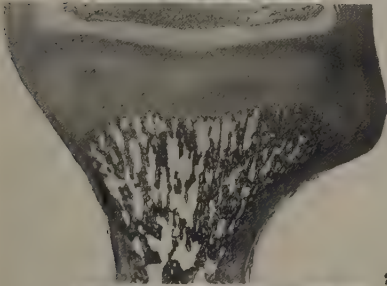
24 Thirteenth caudal vertebra. Pl. B566.



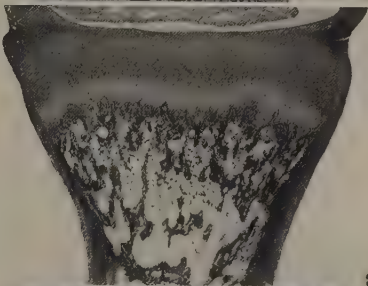
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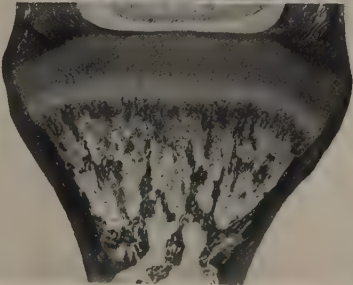
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PLATE 5

EXPLANATION OF FIGURES

Photomicrographs of the epiphyscal region of the third, 8th and 13th caudal vertebrae of rats thyroidectomized at one day of age. Median sagittal sections, H. and E. $\times 17$. Thyroxin, 0.0025 mg daily for 30 days, beginning at 34 days of age. Spec. 9339.

25 Third caudal vertebra. Pl. B567.

26 Eighth caudal vertebra. Pl. B568.

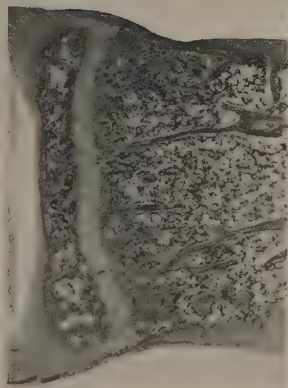
27 Thirteenth caudal vertebra. Pl. B569.

Growth hormone, 0.5 mg, and thyroxin, 0.0025 mg, daily for 30 days, beginning at 34 days of age. Spec. 9353.

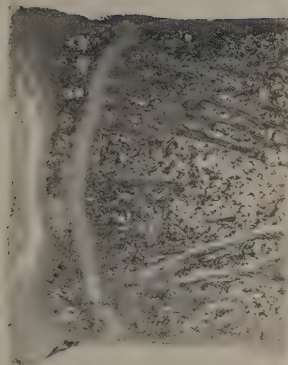
28 Third caudal vertebra. Pl. B570.

29 Eighth caudal vertebra. Pl. B571.

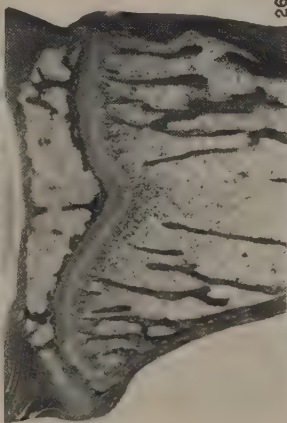
30 Thirteenth caudal vertebra. Pl. B572.



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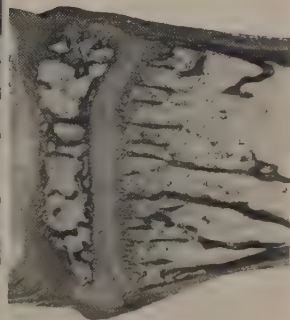
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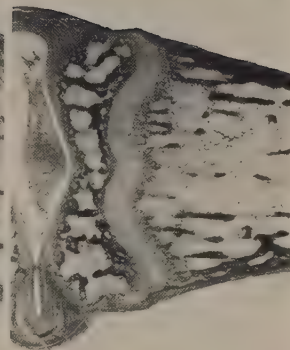
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A TECHNIQUE FOR THE DEMONSTRATION OF THE BLOOD VESSELS IN THE DEVELOPING CENTRAL NERVOUS SYSTEM

ERNST SCHARRER

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FOUR FIGURES

The technical difficulties involved in attempts to demonstrate blood vessels in embryos and fetuses constitute an important limiting factor in the investigation of the development of the cerebral vascular system.

The methods available for the demonstration of blood vessels are based either on the injection of the vessels or on the staining of their content of red blood corpuscles. The injection of blood vessels may be accomplished in two ways. The heart may be used in the living animal to distribute throughout the vascular system a non-toxic dye such as chlorazol sky blue (Campbell, '38) or a colloid solution of mercuric sulfide (Daron, '36). These methods have not been tried by investigators interested in the development of the blood vessels of the central nervous system. More commonly the blood vessels are injected with colored gelatine, latex, etc., under pressure, applied by means of a syringe or a percolator, after the animals have been perfused with saline to remove the blood. These methods, although useful in many instances, have a number of disadvantages which are discussed by Drummond ('44). Quantitative studies, in particular of the pre- and post-natal changes in cerebral vascularity, require complete in-

¹ This paper reports research undertaken under contract with the Office of Naval Research. The conclusions contained in this report are those of the author and do not necessarily reflect the views of the Office of Naval Research.

jections which, as Craigie ('25) and Petré ('38) point out, are difficult to obtain in mammalian embryos and fetuses. Injections with India ink have been successfully practiced by Feeney and Watterson ('46) in chick embryos.

The second type of technique, namely the staining of red cells in the capillaries, has been applied to the blood vessels of the brain by several investigators. The benzidine-nitroprusside method for hemoglobin of Pickworth ('34) and the similar method of Sjöstrand ('34) depend on the uniform filling of the capillaries with red blood corpuscles at the time of death. In order to cause dilation of the capillaries and thus assure their being filled with erythrocytes, Petré ('38) kills the animals by intracardial injection of histamine. Even if it should be possible to achieve uniform filling of the cerebral capillaries by this or similar methods, the subsequent handling of the tissue and loss of blood during removal of the brain is likely to disturb the distribution of the red cells in the cerebral vascular bed. These methods furthermore require frozen sections, the preparations are said not to be permanent, and precipitates around the vessels may disturb the picture. Not all these objections apply to the methods of Altschul ('39) and Eros ('41) who stain the contents of the blood vessels with acid fuchsin in either paraffin, celloidin or frozen sections.

It appears from this brief review that none of these methods is quite satisfactory for the study of the development of the blood vessels of the central nervous system. A new approach was suggested by the observation of Landow, Kabat and Newman ('42) who reported the presence in the adult central nervous system of alkaline phosphatase in the cells of the vascular endothelium. The possibility was explored, therefore, of "staining" selectively the blood vessels of the fetal brain and spinal cord by a method that demonstrates alkaline phosphatase in the endothelium.

MATERIAL AND METHODS

Gomori's ('39) method for the demonstration of alkaline phosphatase in tissues was used in this study. Since we were

interested in the application of this technique as a "staining" method, it was necessary to standardize it so that it could be used in the laboratory as a routine staining method rather than as a histochemical procedure.²

A number of embryos and fetuses of rats and pigs were removed from the uteri and, if small enough, fixed in toto in chilled absolute alcohol. In the case of older specimens the skull was removed in order to permit the effective penetration of the fixing fluid; in still older stages pieces of the brain or the spinal cord were fixed. These pieces were not thicker than 3 or 4 mm in order to assure adequate fixation in the central portions. Apparently one need not be too much concerned in this respect. We obtained, for instance, excellent results in pig embryos which had been dropped in toto into chilled absolute alcohol at the slaughter house and later dissected in the laboratory for further fixation.

The material was kept for 24 to 48 hours in absolute alcohol in the refrigerator. From the alcohol the tissues were transferred to benzene at room temperature for 2 to 3 hours during which time the benzene was changed three times. The tissues were embedded in paraffin (Tissuemat, Fisher) of 56 to 58°C. melting point in an oven at 59°C. in the usual way. It is well to minimize the time during which the tissues are in the oven. A small vacuum oven, operated with a Pressovac pump, is useful in that it permits excellent embedding in less than one hour. The paraffin blocks were kept in the refrigerator until they were cut. The sections were left on the hot plate only long enough to permit flattening; the slides were then left to dry over night at room temperature.

Next the slides were freed of paraffin with three changes of xylene and were brought to water in the usual fashion without coating them with collodion. They were incubated for one and

² I wish here to acknowledge with gratitude the competent cooperation in various phases of this work of Miss JoAnne Sinden, Mrs. Virginia T. Wilson, Mrs. Doris McChesney, Dr. R. Weber and Dr. R. Frandson.

three-quarter hours at 37°C. in a solution prepared in the following manner:

One hundred cubic centimeters of a freshly made 2% solution of sodium glycerophosphate (52% α -, Eastman Kodak Co.) was added to 100 cm³ of a freshly made 2% solution of sodium barbital. Next 200 cm³ distilled water and 20 cm³ of a 2% solution of anhydrous calcium chloride were added. The latter keeps indefinitely and need not be made up freshly each time. Finally 8 cm³ of a 2% magnesium sulfate solution were measured carefully with a pipette and added to the other ingredients. The magnesium sulfate solution will keep indefinitely and need not be prepared freshly each time. The resulting 428 cm³ were sufficient to fill several staining jars so that all slides of a complete series could be processed simultaneously.

After the slides had been incubated, the solution was discarded. The slides were rinsed in distilled water and next immersed 5 minutes in a 2% solution of cobaltous nitrate. This solution was saved and used over and over again. After treatment with cobaltous nitrate the slides were rinsed carefully in several changes of distilled water so that they did not carry any cobaltous nitrate into the next solution, which was an approximately 2% solution of light yellow ammonium sulfide. The sections remained 5 minutes in the ammonium sulfide in which they turned dark. They were then washed in running tap water for 10 to 15 minutes. After washing, the slides were counterstained with toluidin blue or carried directly through the usual series of alcohols and xylene. We passed the slides from the xylene to a jar with perchlorethylene in a well ventilated room (the vapors of perchlorethylene are injurious if inhaled for extended periods of time) and mounted the sections in clarite dissolved in perchlorethylene. This method has been recommended by Gomori for lipase preparations which will fade in xylene media. Mounting in clarite may not be necessary for alkaline phosphatase preparations, but we did not wish to take any chances with damar dissolved in xylene.

The sections used in this study were $15\ \mu$ thick. They show segments of blood vessels of varying length which may be measured for the quantitative determination of the vascularity of a given region as described by Craigie ('20, '24). If it is desired to reconstruct the vascular pattern from a number of sections various methods may be applied. The graphic reconstructions shown in figures 3 and 4 were prepared by drawing the outline of the area to be studied and its blood vessels of 10 consecutive sections with the help of a Leitz (Edinger) projector and superimposing them to obtain one combined drawing. The capillary fragments present in each section supplement each other so that the combined drawing of the capillary network looks much like that of a $150\ \mu$ thick section of brain tissue in which the blood vessels have been injected with India ink. There are several sources of inaccuracies, one of which is the distortion of the periphery of the projected field. However the resulting error can be kept relatively small by drawing only the center portion of the projected area. This method of reconstruction appears adequate to demonstrate the changes in the density and the pattern of the capillaries in different stages of the development of the central nervous system.

RESULTS

In a rat embryo (fig. 1) stained as described above the blood vessels of the brain and spinal cord appear black on a light background since their endothelial cells apparently contain a great deal more alkaline phosphatase than the brain tissue. In this selectivity the capillaries of the brain are unique; the blood vessels of other organs are not stained by the method described above (fig. 1). The significance of this biochemical difference between the blood vessels of the central nervous system and those of other organs cannot be discussed here, but seems interesting enough to deserve further investigation. In all parts of the central nervous system all capillaries stain uniformly (fig. 2). There are some areas where the nervous tissue also contains some alkaline phosphatase and consequently the contrast between blood vessels and nervous tissue

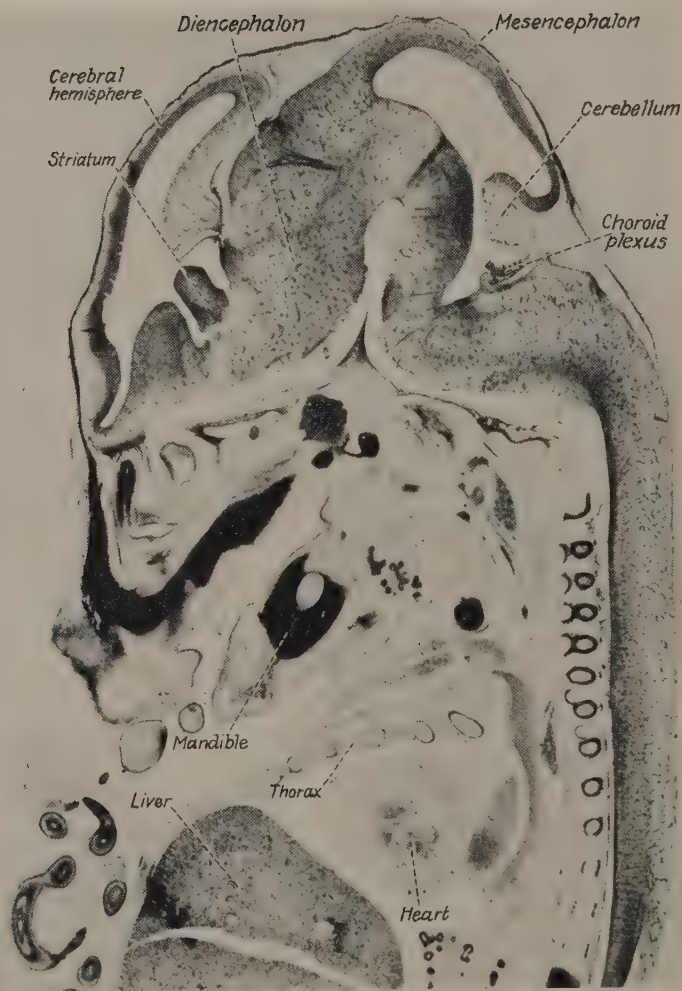


Fig. 1 Sagittal section through a rat embryo of 1.5 cm body length, 15 μ . Gomori's alkaline phosphatase method, photomicrograph. $\times 17$. Note the "staining" of the blood vessels in the brain and spinal cord, and the absence of stained blood vessels in other organs. The developing bones show a strong alkaline phosphatase reaction.

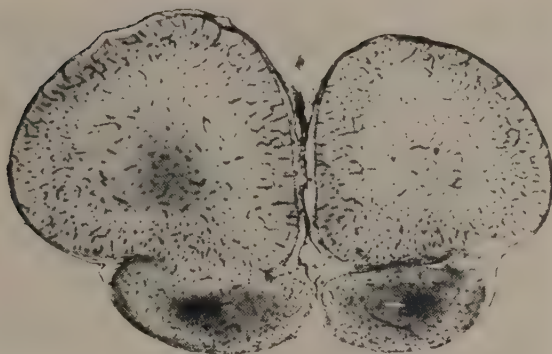


Fig. 2 Cross section through the anterior portion of the cerebral hemispheres of a rat fetus of 3.8 cm body length, 15μ . Gomori's alkaline phosphatase method, photomicrograph. $\times 20$. The blood vessels are selectively "stained."



Fig. 3 Graphic reconstruction of the capillary bed of the cornu ammonis and cortex of a rat embryo of 1.5 cm body length. The drawing was made from 10 consecutive sections of 15μ each, and represents, therefore, the capillary bed of a corresponding section of 150μ thickness (see text p. 323).

is less sharp. However, this does not interfere with the study of the architecture and density of the capillary bed throughout the central nervous system.

The cornu ammonis has been selected as an example (figs. 3 and 4) to illustrate the differences in capillary density in two different stages of development. A comparison of the vascular differentiation of the archicortex with that of the neocortex in the two stages of development confirms the observations of Craigie ('31) who found no precocious development of the blood supply of the cornu ammonis.

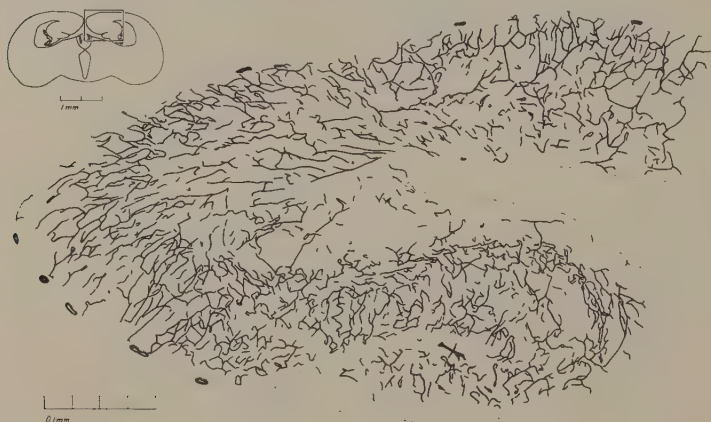


Fig. 4 Graphic reconstruction of the capillary bed of the cornu ammonis and cortex of a newborn rat of 4.6 cm body length. Technique as in figure 3. Note the density and differentiation of the capillary bed in comparison to that shown in figure 3.

SUMMARY

The use of Gomori's method for the demonstration of alkaline phosphatase in tissues is recommended for the study of the development of cerebral blood vessels. Since this method "stains" the endothelium of the blood vessels, the completeness with which the capillary bed of the fetal central nervous system may be demonstrated does not depend upon the patency of the vessels.

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ELECTRON MICROGRAPHS OF THE AMPHIASTER IN THE WHITEFISH BLASTULA (COREGONUS CLUPERFORMIS)^{1, 2}

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NINE FIGURES

The architecture of the amphiaster has been and still remains an important problem in cytology. This is due, in part, to the fact that the elements of the aster and spindle cannot be clearly seen in most living cells. Accordingly, the fibrous appearance of the amphiaster often observed in fixed and stained preparations has been held as due to artifact induced by the fixation process. (For discussion of this point see Wilson, '37 and Fry-Wyssling, '48.) On the other hand, considerable evidence has recently accumulated to show that in certain living cells a typical fibrous appearance of the spindle and asters are present (Cleveland, '35). In addition, Schmidt ('36a, b), as well as others, have conclusively shown that both the components of the spindle and those of the aster, under polarized light, show birefringence in their long axis which means that they are composed of parallel oriented protein molecules. Other evidence derived from centrifuging and treatment with hypertonic solutions support the classical concept concerning the reality of the fibrous nature of the spindle and asters. Such evidence has led Schrader ('44) to

¹ We are indebted to Professor C. D. Janney of the Radiation Research Laboratory, State University of Iowa, for aid in the operation of the electron microscope.

² This investigation has been aided by a grant from the Iowa Division of the American Cancer Society, and by a grant from the United States Public Health Service (administered by Professor J. H. Bodine).

conclude that "The question of the reality of spindle fibers may be regarded as settled."

It is the purpose of this paper to present an enlarged view of the elements of the amphister as seen in electron micrographs. Since the material for this study had to be fixed and dried it obviously does not bear on the problem above mentioned, namely the possibility of extensive artifact induced upon fixation. We are accepting the view that what is seen here represents at least roughly the condition existing in the living cell. However, this must not be taken to infer that some degree of distortion has not occurred as will be discussed below.

MATERIAL AND METHODS

The material for this study consists of the developing blastula of the whitefish, kindly supplied us by the General Biological Supply House, Inc., Chicago, Illinois. The blastulae were fixed in a mixture of 90 cm³ of 70% ethyl alcohol, 5 cm³ of glacial acetic acid and 10 cm³ of formaldehyde. They were subsequently embedded in 62°C. melting point "tissuemat" and sectioned at 0.2 to 0.5 μ by the aid of a no. 829 Spencer thin section adapter for the 820 Spencer rotary microtome. A special copper collar was fitted to the back of the metal microtome plug in such a way that ice water could be kept continually flowing through it. Thus, by conduction the mounted paraffin block containing the tissue could be kept at approximately 14°C. For sectioning, a Spencer blade holder equipped with a "Valet" razor blade was used. In front of the blade was attached a small glass well filled with an ice-chilled mixture of 45% ethyl alcohol and 2% glycerine. This adaptation was found to be very useful in keeping the sections from jamming on the blade and greatly facilitated their handling. The sections were spread on water at 45°C. and mounted on the conventional celloidin covered grids. When dry the paraffin was removed by two changes of xylol. No stains were added to the cells.

The electron microscope used was an R.C.A. Model E.M.U. equipped with an unbiased gun. Magnification of the electron micrographs is indicated by the micron scale mounted on each figure.

Terminology

As Schrader ('44) points out, the majority of the evidence indicates the presence of the following components associated with the mitotic figure: (1) "continuous fibers" connecting the two centers or poles; (2) "chromosomal fibers" which are connected with the kinetochore of the chromosomes and usually extend to the poles; (3) "interzonal fibers" extending between the separating chromosomes in anaphase and telophase; (4) center or pole, a morphologically distinguishable body toward which the spindle fibers and astral rays are oriented, which may or may not contain a centriole; (5) chromosomes, complex bodies, some or all of which may participate in the mitotic process.

The mitotic figures of dividing whitefish blastulae as seen through the light microscope are too well known to warrant a detailed description of them here. Excellent photographs of the amphiaster in this material may be found in catalog no. 17 of the General Biological Supply House, Inc., Chicago, Illinois, or in Sharp ('34). We chose to study the dividing whitefish blastula because of its striking asters and well developed spindle. However, no centriole is visible in either of the asters. The fixed cytoplasm gives the appearance of a fibrous network.

Electron micrographs of dividing cells

A. Spindle. It should be pointed out that electron micrographs of non-dividing cell protoplasm show it to be a relatively unoriented fibrous network. This condition probably represents the coagulated "structural proteins" within the cell. Presumably certain of the "mobile proteins" as well as the lipid containing components have for the most part been removed by the method of preparation.

In figure 1 is represented what appears to be a metaphase amphaster. However, a distinct doubling of the chromosomes is not evident. An enlarged view of a portion of the equatorial plate of this cell is illustrated in figure 3. Here relatively large distinct oriented fibers are clearly seen. The fibers vary in size from 1,000 to 3,000 Å and appear continuous. Smaller interconnecting fibers are also evident in many parts of the spindle. Such a condition is in harmony with the view that the spindle consists of a jelled region. In these figures it will be observed that it is not possible to distinguish clearly (by a study of the morphology alone) between the various types of spindle fibers, i.e., "chromosomal" and "continuous." Neither is it possible to observe the kinetochore of the chromosomes.

The identity of a short portion of a "chromosomal fiber" seems definite in figure 4. However, here as in the other figures, no details of its attachment to the chromosome can be made out. Unpublished data on other types of dividing cells show promise of possible analysis of this point with the electron microscope. It is interesting to note that the spindle fibers do not vary greatly in size and are clearly oriented in the direction of the poles. Such a condition argues for the view that both the "chromosomal" and "continuous" fibers are structurally fundamentally the same. That is, both are derived from the fibrous micellar hyaloplasm by an orientation of its ultrastructure. How certain of these fibers ("chromosomal") become attached to the chromosomes is not clear from these figures.

B. Anaphase. Figures 2 and 5 show stages in which the chromosomes are nearing the astral center. Typical large fibrous oriented spindle elements are present. In fact, figuratively speaking this condition of the fibers looks like an elongated "brush heap," to use the expression of Seifriz ('36). Between the larger orienting fibers may be seen smaller interconnecting strands. Figure 5 especially demonstrates how the spindle elements lose their orientation at the periphery of the central portion of the aster. Many of the

spindle fibers appear beaded, or banded, a condition which is possibly due to irregular contractions at the time of fixation (fig. 3). Efforts thus far have failed to disclose a regular periodic banding of the fibers of the same relative order of spacing like that demonstrated for collagenous fibers. However, further work may eventually demonstrate their presence.

In figure 2 a more advanced stage of mitosis is present. Here certain of the chromosomes appear to be breaking down as is evidenced by the lighter and darker areas present within them. The spindle elements show evidence of degeneration as noted by their less regular alignment, robust appearance and by the apparent interruption of certain of them. Certainly by this time a solution of the spindle is occurring and here may be evidence as above described of the morphological changes accompanying this process.

Asters. It is relatively easy to identify the central region of the asters on the fluorescent screen because of their relatively dense reticular sponge-like structure (figs. 5, 6). This dense reticulum differs from any other region of the cell. In well developed asters the rays appear structurally similar to those of the spindle elements except that they vary more in diameter and in some cases appear to be discontinuous or broken. The appearance of a small portion of an aster (two rays) extending from their center or origin to their attachment at the cell membrane is illustrated in figures 6, 7, 8, 9. In this composite picture figures 6 and 7 do not exactly overlap in all regions. However, as the rays emerge from their astral focal point they are oriented for the most part toward the cell membrane (bottom of fig. 9). They are not smooth but irregularly banded, a condition possibly due to contraction induced upon fixation or drying. The interconnecting fibers are more numerous here than they are where the astral rays approach the surface of the cell. More centrifugally the astral rays clearly appear more widely separated and to be composed of one or more fibers (figs. 8, 9). In the cortex region they still remain relatively widely separated and seem to be

associated with more irregular dense masses of coagulated material. The rays are definitely attached to the membrane of the cell (bottom of fig. 9).

How nearly these figures of the aster represent the condition in the living cell cannot be stated. Some degree of artifact undoubtedly exists but it should be remembered that both Heilbrunn ('20) and Monné ('44) have presented good evidence that the astral rays are fibrous.

It is conceivable, as pointed out by Chambers ('24), that the clear spaces between the fibers here illustrated might represent regions where oriented cytoplasmic flow could occur.

DISCUSSION

It is obvious that the electron micrographs presented here of the amphiaster substantiate the view that it is composed of fibers, a condition long ago described by many cytologists. It was not possible here to differentiate on a structural basis a difference between the "chromosomal" and "continuous" fibers. Only in those cases where the "chromosomal fibers" were observed to be attached to the chromosomes could their identity be determined with certainty. However, unpublished data indicate that the identity of the various types of spindle fibers may be easily established in other types of dividing cells. In fact, the chromosomal fibers of certain crayfish cells appear to be composed of smaller fibrous elements. However, we are inclined, for the present, to view the elements of the amphiaster in the whitefish blastula, i.e., "continuous fibers," "chromosomal fibers," and possibly astral rays, as morphologically the same except for size. In other words, they all appear to be derived from the basic ground cytoplasm which is composed of an anastomosing protein meshwork which becomes oriented under the influence of the astral centers. This condition is undoubtedly associated with the change in viscosity of the cell that is known to occur at this time.

In other words, the work here reported seems completely in accord with the view expressed by F. O. Schmitt ('39, '40) that "The results of the polarization and x-ray optical analy-

sis are in agreement with the view that animal fibers, whether in large compact bundles (muscle, tendon), or microscopic and intracellular (chromosomes, spindle and astral fibers) are constructed of an anastomosing meshwork of sub-microscopic fibrous particles or micelles oriented with long axes parallel to the fiber axis. . . . Data on extensility and viscosity require that the particles be interlinked by covalent strands such as compose the particles themselves, although the greater fraction of the strands are longitudinally oriented." Monné ('44) found by using polarized light on the living sea urchin egg that it, too, was composed of fibrils made up of bundles of polypeptide chains which join in the form of a texture. It is known that fibers such as collagen when treated with acid, or with pepsin, fragment longitudinally into fine filaments which retain their periodicity in the axial direction. They may even be made to pass through filter paper, and upon neutralization of the medium under certain conditions will reorganize themselves into new fibrillae with a periodic axial structure (De Robertis, Nowinski and Saez, '48). Even some proteins in globular form such as insulin or actin, a protein extracted from muscle, may under suitable conditions be reversibly transformed into a fibrous state. It is conceivable as pointed out by Schmitt ('39), Lazarow ('43) and others that some such mechanism may be operating in the formation and destruction of the asters and spindle fibers. Such a condition could conceivably be controlled by special enzyme systems.

We were not successful in observing periodic banding in either the astral or spindle fibers like that so beautifully demonstrated for collagen (Schmitt and Gross, '48). However, further work using electron strains, shadow casting and mitotic poisons may eventually demonstrate their presence.

It seems unnecessary to discuss here the numerous papers dealing with the spindle fibers since, as previously mentioned, an excellent review of this subject has recently been published by Schrader ('44). We are well aware that considerable variation may exist in spindle elements in different types of cells.

Accordingly we wish to make no claim that what we have reported here for dividing whitefish blastula will necessarily hold for all types of amphiasters.

SUMMARY

Electron micrographs of dividing whitefish blastulae have been made. Certain adaptations for thin sectioning such as a method for cooling the block and for removing the sections from the knife have been described.

The amphiaster elements such as "chromosomal fibers," "continuous fibers," and astral fibers have been observed, oriented in the same manner as described for them under the light microscope. In the whitefish the spindle elements are nearly the same size and seem to be constructed in the same way. In other words, it was difficult to identify one type spindle fiber from the other except in those cases where their attachments could be established. They often showed a gross but no periodic banding of their ultrastructure. Inner connecting strands, that is, smaller fibers extending between the more grossly oriented spindle elements, were observed.

The astral rays appear fibrous and oriented from the dense reticular center toward the periphery of the cell. They may or may not appear continuous, probably depending on whether or not they have been broken by the technique of preparation. Numerous smaller unoriented fibers extend between the astral rays.

The ground cytoplasm has a sponge-like appearance. This is apparently the protein component of the cytoplasm commonly referred to in the literature as "structural" or "framework cytoplasm."

From the existing literature on fibrous proteins it is conjectured that the asters and spindle elements are derived from the structural cytoplasmic framework by orientation of its ultrastructure much in the same way that others have demonstrated for the organization and disorganization of certain other noncellular fibrous protein elements.

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PLATE 1

EXPLANATION OF FIGURES

1 Metaphase showing chromosomes, "chromosomal fibers," "continuous fibers" and asters. Scale: $10\ \mu$.

2 Late anaphase. Here the elements of the spindle show evidence of disorganization by their less sharp orientation toward the poles, apparent discontinuity of some of them and in general by their less robust appearance as compared to figure 1. Scale: $2\ \mu$.

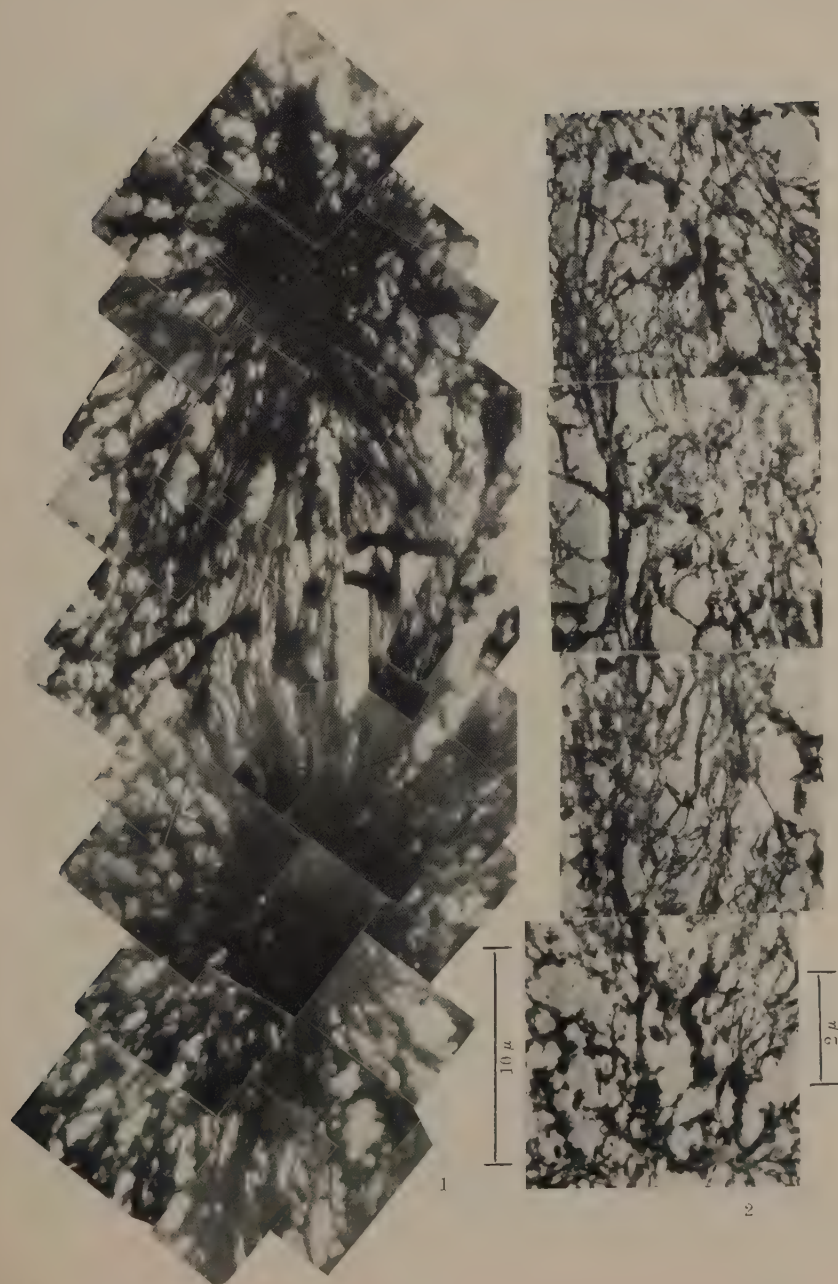


PLATE 2

EXPLANATION OF FIGURE

3 Enlarged view of portion of metaphase plate of figure 1. The dense body in center is a chromosome, about which are seen the spindle fibers. Scale: 1μ .

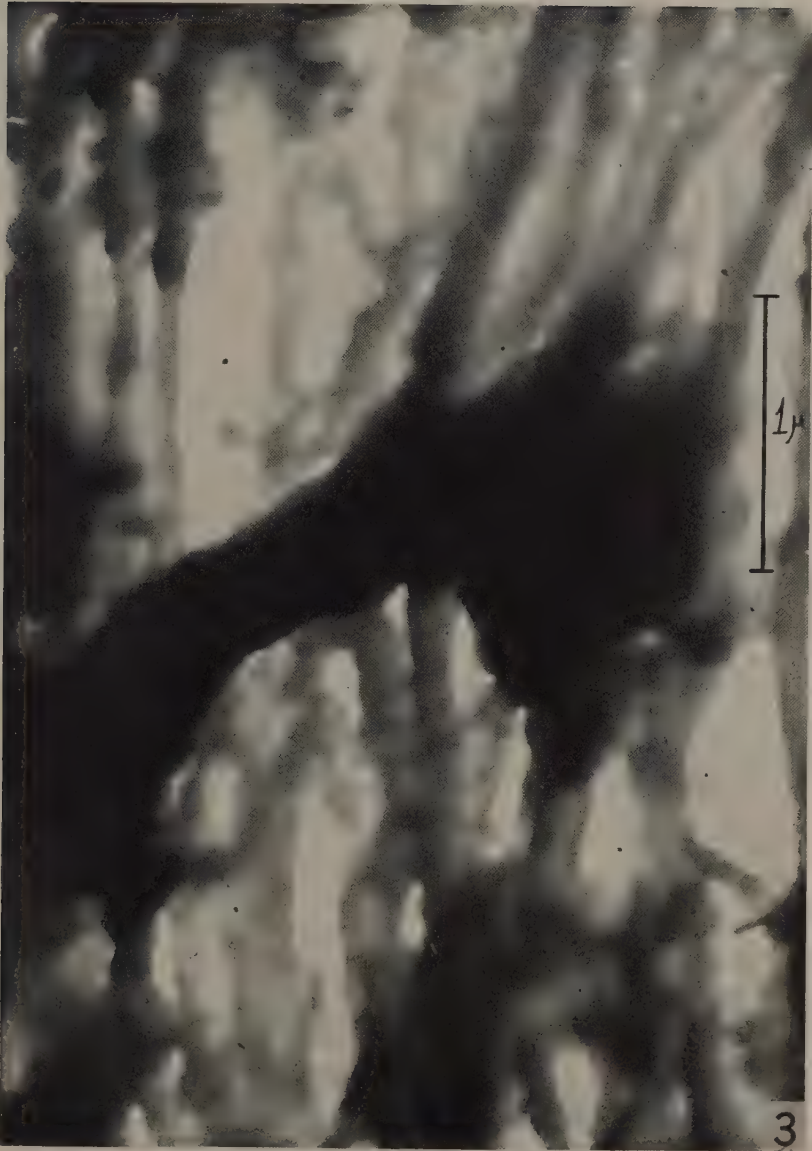


PLATE 3

EXPLANATION OF FIGURES

- 4 Chromosome with fiber attached.
- 5 Anaphase showing spindle fibers and center of aster. Here the difference in organization of the astral center and rays may be seen clearly. Scale: 1 μ .



PLATE 4

EXPLANATION OF FIGURES

6, 7, 8 and 9 Consecutive figures of a portion of aster showing its organization from center (fig. 6) to its attachment to cell membrane at bottom of figure 9. Scale: 1μ .

